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(54) Title: ANTIVIRAL TRANSGENIC PLANTS, VECTORS, CELLS AND METHODS

(57) Abstract

Isolated 2-5A-dependent RNases, an interferon-induced enzyme which is activated by 5'-phosphorylated, 2',5'-linked oligoadenylates (2-5A) and implicated in both the molecular mechanisms of interferon action and in the fundamental control of RNA stability in mammalian cells, and encoding sequences therefor are disclosed. The expression cloning and analysis of murine and human 2-5A-dependent RNases is also disclosed. In addition, recombinant nucleotide sequences, recombinant vectors, recombinant cells and antiviral plants which express, for example, 2-5A-dependent RNase, 2-5A synthetase and/or double-stranded RNA dependent protein kinase (PKR), or other amino acid sequences which have activity that interferes with or inhibits viral replication are disclosed.

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ANTIVIRAL TRANSGENIC PLANTS, VECTORS, CELLS AND METHODS

Related Applications

This application for U.S. patent is a continuation-in-part of U.S. patent application, which was assigned Serial No. 08/028,086 and filed on March 8, 1993.

Field of the Invention

The present invention relates to isolated 2-5A-dependent RNases having the ability to bind 2-5A and/or cleave single stranded RNA when bound to 2-5A, encoding sequences therefor, recombinant nucleotide molecules, recombinant vectors, recombinant cells, and antiviral transgenic plants which express, for example, antiviral animal amino acid sequences which have activity similar or identical to 2-5A-dependent RNase, 2-5A synthetase and/or PKR.

Background

Control of RNA degradation is a critical cell function, and gene expression is often regulated

at the level of RNA stability. See, e.g., Shaw, G. and Kamen, R., Cell, 46:659-667 (1986). Nevertheless, relatively little is known about the biochemical pathways that mediate RNA degradation in mammalian or plant systems. For instance, most if not all of the ribonucleases responsible for mRNA turnover in mammalian or plant cells remain unidentified. This was reviewed in Brawerman, G., Cell, 57:9-10 (1989).

Presently, the 2-5A system is believed to be the only well-characterized RNA degradation pathway from higher animals including man. See FIG. 1. See also, e.g., Kerr, I.M. and Brown, R.E., Prod. Natl. Acad. Sci. U.S.A., 75:256-260 (1978) and Cayley, P.J. et al., Biophys Res. Commun., 108:1243-1250 (1982); reviewed in Sen, G.C. and Lengyel, P., J. Biol. Chem., 267:5017-5020 (1992). The activity of the 2-5A system is believed to be mediated by an endoribonuclease known as 2-5A-dependent RNase. See Clemens, M.J. and Williams, B.R.G., Cell, 13:565-572 (1978). 2-5A-dependent RNase is a unique enzyme in that it requires 2-5A, unusual oligoadenylates with 2',5' phosphodiester linkages, $p_n(A2'p)_nA$, for ribonuclease activity. See Kerr, I.M. and Brown, R.E., Prod. Natl. Acad. Sci. U.S.A., 75:256-260 (1978). 2-5A is produced from ATP by a family of synthetases in reactions requiring

double-stranded RNA (dsRNA). See FIG. 1. See also Hovanessian, A.G. et al., Nature, 268:537-539 (1977); Marie, I. and Hovanessian, A.G., J. Biol. Chem., 267:9933-9939 (1992). 2-5A is unstable in cells and in cell-free systems due to the combined action of 2',5'-phosphodiesterase and 5'-phosphatase. See Williams, B.R.G. et al.; Eur. J. Biochem., 92:455-562 (1978); and Johnson, M.I. and Hearl, W.G., J. Biol. Chem., 262:8377-8382 (1987). The interaction of 2-5A-dependent RNase and 2-5A(K_d = 4 X 10^{-11} M), Silverman, R.H. et al., Biol. Chem., 263:7336-7341 (1988), is highly specific. See Knight, M. et al., Nature, 288:189-192 (1980). 2-5A-dependent RNase is believed to have no detectable RNase activity until it is converted to its active state by binding to 2-5A. See Silverman, R.H., Anal. Biochem., 144:450-460 (1985). Activated 2-5A-dependent RNase cleaves single-stranded regions of RNA 3' of UpNp, with preference for UU and UA sequences. See Wreschner, D.H. et al., Nature, 289:414-417 (1981a); and Floyd-Smith, G. et al., Science, 212:1020-1032 (1981). Analysis of inactive 2-5A-dependent RNase from mouse liver revealed it to be a single polypeptide of approximately 80 kDa. See Silverman, R.H. et al., Biol. Chem., 263:7336-7341 (1988).

Although the full scope and biological significance of the 2-5A system remains unknown,

studies on the molecular mechanisms of interferon action have provided at least some of the functions. Interferons α, β or Y are believed to induce the accumulation of both 2-5A-dependent RNase, Jacobsen, H. et al., Virology, 125:496-501 (1983A) and Floyd-Smith, G., J. Cellular Biochem., 38:12-21 (1988), and 2-5A synthetases, Hovanessian, A.G. et al., Nature, 268:537-539 (1977), reviewed in Sen, G.C. and Lengyel, P., J. Biol. Chem., 267:5017-5020 (1992). Furthermore, several investigations have implicated the 2-5A system in the mechanism by which interferon inhibits the replication of picornaviruses. Indeed, 2-5A per se and highly specific 2-5A mediated rRNA cleavage products were induced in interferon-treated, encephalomyocarditis virus (EMCV)-infected cells. See Williams, B.R.G., Nature, 282:582-586 (1979); Wreschner, D.H. et al., Nucleic Acids Res., 9:1571-1581 (1981b); and Silverman, R.H. et al., Eur. J. Biochem., 124:131-138 (1982a). In addition, expression of 2-5A synthetase cDNA inhibited the replication of picornaviruses, Chebath, J., Nature, 330:587-588 (1987) and Rysiecki, E.F. et al., J. Interferon Res., 9:649-657 (1989), and the introduction of a 2-5A analogue inhibitor of 2-5A-dependent RNase into cells reduced the interferon-mediated inhibition of EMCV replication. See Watling, D. et al., EMBO J., 4:431-436 (1985).

Further, 2-5A-dependent RNase levels were correlated with the anti-EMCV activity of interferon, Kumar, R. et al., J. Virol., 62:3175-3181 (1988), and EMCV-derived dsRNA both bound to and activated 2-5A synthetase in interferon-treated, infected cells. See Gribaudo, G. et al., J. Virol., 65:1948-1757 (1991).

The 2-5A system, however, almost certainly provides functions beyond the antipicornavirus activity of interferons. For instance, introduction of 2-5A into cells, Hovanessian, A.G. and Wood, J.N., Virology, 101:81-90 (1980), or expression of 2-5A synthetase cDNA, Rysiecki, G. et al., J. Interferon Res., 9:649-657 (1989), inhibits cell growth rates. Moreover, 2-5A-dependent RNase levels are elevated in growth arrested cells, Jacobsen, H. et al., Proc. Natl. Acad. Sci. U.S.A., 80:4954-4958 (1983b), and 2-5A synthetase, Stark, G. et al., Nature, 278:471-473 (1979), and 2-5A-dependent RNase levels are induced during cell differentiation. See, e.g., Krause, D. et al., Eur. J. Biochem., 146:611-618 (1985). Therefore, interesting correlations exist between 2-5A-dependent RNase and the fundamental control of cell growth and differentiation suggesting that the 2-5A system may function in general RNA metabolism. The ubiquitous presence of the 2-5A system in reptiles, avians and mammalians certainly

supports a wider role for the pathway. See, for example, Cayley, P.J. et al., Biochem. Biophy. Res. Commun., 108:1243-1250 (1982).

While it is presently believed that the 2-5A system is the only well-characterized RNA degradation pathway from higher animals, the dsRNA-dependent protein kinase enzyme, known as PKR, is also thought to have antiviral effects in higher animals. Like the 2-5A synthetase enzyme, it is believed that PKR is stimulated by dsRNA. It is believed that activated PKR phosphorylates the alpha subunit of translation factor eIF_2, known as eIF_2-alpha, which indirectly inhibits protein synthesis initiation. It is believed that interferons α, β, and γ induce the accumulation of PKR. See Hoavanessian et al.: J. Interferon Res., 9:641-647 (1989).

Like the 2-5A system, the PKR system is also likely to provide functions beyond the antipicornavirus activity of interferons. See Meurs, E.F. et al.: J. Virology, 66:5805-5814 (1992). For example, expression of mutant forms of PKR in NIH 3T3 cells resulted in tumor formation when injected into nude mice. See Meurs, E.F. et al.: Proc. Natl. Acad. Sci U.S.A., 90:232-236 (1993).

In short, the 2-5A system and the PKR system inhibit viral protein synthesis. This is

believed to be accomplished by the 2-5A system by degrading mRNA and rRNA whereas the PKR system is believed to accomplish this by indirectly inhibiting protein synthesis initiation.

Viral plant diseases are pandemic and their severity varies from mild symptoms to plant death. The majority of plant viruses are believed to have single stranded RNA genomes. Moreover, it is currently believed that plants are void of the three enzymes discussed above, i.e., PKR, 2-5A synthetase and 2-5A-dependent RNase. See Cayley, P.J. et al.: Biochem. Biophys Res. Commun., 108:1243-1250 (1982) and Devash, Y. et al.: Biochemistry, 24:593-599 (1985); but see Crum, C. et al.: J. Biol. Chem., 263:13440-13443 (1988); Hiddinga, H.J. et al.: Science, 241:451-453 (1988); Sela, I.: TIBS, pp. 31-33 (Feb 1981); and Devash, Y. et al.: Science, 216:1415-1416.

Notwithstanding the importance of 2-5A-dependent RNase to the 2-5A system, 2-5A-dependent RNase enzymes having ribonuclease function have not been isolated, purified or sequenced heretofore. Consequently, there is a demand for isolated, active 2-5A-dependent RNases and their complete amino acid sequences, as well as a demand for encoding sequences for active 2-5A-dependent RNases. There is also a

demand for plants which are resistant to viruses such as the picornaviruses.

Summary of the Invention

In brief, the present invention alleviates and overcomes certain of the above-mentioned problems and shortcomings of the present state of the art through the discovery of novel, isolated 2-5A-dependent RNases and encoding sequences therefor.

Broadly speaking, the novel 2-5A dependent RNases of the instant invention are involved in the fundamental control of single stranded RNA decay in animal cells, such as mammals, and are also present in animal cells, such as avian and reptilian cells. More particularly, the novel 2-5A dependent RNases of the present invention have the ability to degrade single stranded RNA, mainly 3′ of UpUp or UpAp sequences, after they are activated by binding to 5′-phosphorylated,2′,5′-linked oligoadenylates (hereinafter "2-5A"). As a result, it is believed that the novel 2-5A dependent RNases are useful in connection with inhibition of cell growth rates, viral replication and in connection with interferon treatment of viral infection and cancer. As used herein, the term "2-5A-dependent RNase(s)" is used in a broad sense and is meant to include any amino acid sequence which includes a 2-5A binding domain and/or

ribonuclease function when the 2-5A-dependent RNase is activated by 2-5A.

The novel 2-5A dependent RNases of the present invention are protein enzymes having molecular weights on the order of between about 74 KDa (murine) and about 84 KDa (human), as determined by gel electrophoresis migration and/or prediction from their respective encoding nucleotide sequences. For example, a human 2-5A-dependent RNase of the instant invention has a molecular weight of about 83,539 Da as determined from the amino acid sequence predicted from the encoding sequence therefor, whereas the murine 2-5A-dependent RNase has a molecular weight of about 74 KDa as determined by gel electrophoresis migration and from prediction of the amino acid sequence from the encoding sequence. While an about 74 KDa molecular weight is reported herein for a murine 2-5A-dependent RNase, it should nevertheless be appreciated that the reported molecular weight is for an incomplete murine 2-5A-dependent RNase. It is nevertheless believed that once completely sequenced, i.e., when an about 84 amino acid end region is identified, the molecular weight of a complete murine 2-5A-dependent RNase will be similar to that of human, i.e., about 84 KDa.

It should also be readily apparent to those versed in this art, however, that since gel electro-

phoresis migration has been employed to determine molecular weight of a murine 2-5A-dependent RNase, the 74 KDa molecular weight is only an estimate based upon relative migration.

The amino acid sequence for human 2-5A-dependent RNase protein is depicted in FIG. 3 and Table 1. The encoding sequence for the human 2-5A-dependent RNase protein is also set forth in Table 1. The mRNA for human 2-5A-dependent RNase is about 5.0 Kb in size. The virtually complete amino acid sequence for the murine 2-5A-dependent RNase protein and the encoding sequence therefore is depicted in Table 2. The mRNA for murine 2-5A-dependent RNase is about 5.7 Kb in size.

Analysis of the amino acid sequences of the 2-5A-dependent RNases of the present invention have revealed several characteristics unique to the 2-5A-dependent RNases. For example, it has been discovered that the novel 2-5A dependent RNases of the instant invention include the following unique domains which span between the amino terminus and the carboxy terminus. For instance, it has been discovered that there are at least four and possibly as many as nine or more ankyrin repeats, of which three lie closest to the amino terminus. However, while four ankyrin repeats have been discovered, it is believed that there may be additional ankyrin

repeats that may total, for instance, about eight or more when the amino acid sequences of the 2-5A-dependent RNases of the present invention are further analyzed. It is believed that these ankyrin repeats may possibly function in protein-protein interaction. Ankyrin repeat 1 generally lies between amino acids designated as 58-90 in Tables 1 and 2. Ankyrin repeat 2 generally lies between amino acids designated as 91-123 in Tables 1 and 2. Ankyrin repeat 3 generally lies between amino acids designated as 124-156 in Tables 1 and 2. Ankyrin repeat 4 generally lies between amino acids designated as 238 and 270 in Tables 1 and 2. See also FIGS. 10A and 10B.

It has also been discovered that the novel 2-5A dependent RNases include a cysteine rich region (which has homology to zinc fingers) that lies closer to the carboxy terminus than the amino terminus which may possibly function in RNA recognition or in formation of protein dimers. The cysteine rich region is believed to include about 5 or 6 cysteine residues which generally lie between amino acids designated as 395-444 in the human sequence as reported in Table 1 and FIG. 4, or between amino acids designated as 401-436 in the murine sequence as reported in Table 2 and FIG. 4.

Still further, it has been discovered that the novel 2-5A dependent RNases include a duplicated phosphate binding (2 P-loops) motif which lies generally within the ankyrin repeat motifs. It is believed that the two P-loops are in the same orientation and constitute the binding domain necessary for binding 2-5A. It is further believed that each P-loop motif includes a lysine residue which is essential for maximum 2-5A binding activity. The lysine residues are designated as 240 and 274 in Tables 1 and 2.

It has been further discovered that the 2-5A-dependent RNase proteins contain an amino acid region which follows the cysteine rich region that is believed to be homologous to protein kinases. Within this region, there is believed to be separate domains designated as domains VI and VII which generally lie between amino acid residues designated as 470-504 in Tables 1 and 2. More particularly, as to the human sequence of 2-5A-dependent RNase, domain VI generally lies between amino acid residues designated as 471-491 and domain VII generally lies between amino acid residures designated as 501-504, as reported in Table 1 and FIG. 4. As to the murine sequence of the 2-5A-dependent RNase, domain VI generally lies between amino acids designated as 470-489 and domain

VII generally lies between amino acid residues designated as 499-502, as reported in Table 2 and FIG. 4.

It has also been discovered that there is limited homology between the amino acid sequences for the 2-5A-dependent RNases of the present invention and RNase E, encoded by the altered mRNA stability (ams)/rne gene of E. Coli. Uniquely, the limited homology is generally conserved between the murine and human amino acid sequences for 2-5A-dependent RNases and generally lies between a 200 amino acid region. More particularly, for the human sequence, the amino acid region spans amino acid residues designated as 160-349 in Table 1 and FIGS. 9A and 9B. With respect to the murine sequence, the amino acid region spans amino acid residues designated as 160-348 in Table 2 and FIGS. 9A and 9B.

It has been further discovered and is believed that almost the entire, if not complete, amino acid sequences of the novel 2-5A-dependent RNase proteins of the instant invention are necessary for ribonuclease function. For example, it is believed that, when an about 84 amino acid region at the carboxy terminus is present in the human 2-5A-dependent RNase, the human 2-5A-dependent RNase has ribonuclease function in the presence of 2-5A. In contrast, when the murine 2-5A-dependent RNase

lacks the about 84 amino acid region at the carboxy terminus, it lacks ribonuclease function.

With respect to the binding activity of a murine 2-5A-dependent RNase protein to 2-5A, it has been discovered that when one P-loop is deleted from the repeated P-loop motif of a murine 2-5A-dependent RNase protein, nearly all 2-5A binding activity is lost, and that when both P-loops are deleted, virtually complete activity is lost. However, it has been found that, even though the carboxy terminus portion of the amino acid sequence of a murine 2-5A-dependent RNase protein following the repeated P-loop motif has been deleted, partial 2-5A binding activity is maintained.

It has been further discovered that when lysine residues 240 and 274 are replaced with asparagine residues in both P-loop motifs, significant 2-5A binding activity of a murine 2-5A-dependent RNase protein is lost. It has been further discovered, however, that when either lysine residue 240 or 274 is replaced in either P-loop motif, only partial 2-5A binding activity is lost. It is therefore believed that the presence of both P-loop motifs in the amino acid sequences for the 2-5A dependent RNases of the present invention plays an important role in 2-5A binding activity. It is further believed that the presence of lysine residues

240 and 274 in each P-loop motif plays an important role for enhanced 2-5A binding activity. It is also believed that the presence of virtually the entire amino acid sequence of the 2-5A-dependent RNases of the present invention provides for even further enhanced 2-5A binding activity, as well as provides for ribonuclease function.

In addition, the present invention relates to the cloning of murine and human 2-5A-dependent RNases and novel murine and human clones. Recombinant and naturally occurring forms of 2-5A-dependent RNase displayed virtually identical 2-5A binding properties and ribonuclease specificities.

The present invention further contemplates the use of the novel isolated, 2-5A-dependent RNases and encoding sequences therefor, as well as analogs and active fragments thereof, for use, for instance, 1.) in gene therapy for human and animal diseases including viral disease and cancer, 2.) as genetic markers for human disease due to perhaps cancer or viral infection, 3.) to develop plants and animals resistant to certain viruses, and 4.) as enzymes in connection with research and development, such as for studying the structure of RNA. In one manner to accomplish the above, and as contemplated by the present invention, the encoding sequences of the

instant invention may be utilized in ex vivo therapy, i.e., to develop recombinant cells using the encoding sequence of the present invention using techniques known to those versed in this art. In another manner which may be employed to accomplish the above, the encoding sequences of the present invention may be combined with an appropriate promoter to form a recombinant molecule and inserted into a suitable vector for introduction into an animal, plant, or other lower life forms also using techniques known to those skilled in this art. Of course, other suitable methods or means known to those versed in this art may be selected to accomplish the above-stated objectives or other objectives for which the novel 2-5Ã-dependent RNases and encoding sequences of the present invention are suited.

The present invention also contemplates novel transgenic plants, as indicated above, which are resistant to viruses such as the picornaviruses. Generally speaking, the transgenic plants of the present invention include any inserted nucleotide sequence encoding any type of antiviral amino acid sequence, including proteins. Preferably, the antiviral nucleotide sequences introduced into plants in accordance with the present invention are animal antiviral genes, such as those genes which are stimulated in response to interferon production

and/or treatment. These include, for example, those animal antiviral genes that encode 2-5A-synthetase, 2-5A-dependent RNase, and PKR. These interferon-regulated proteins, 2-5A-synthetase, 2-5A-dependent RNase and PKR (the dsRNA-dependent protein kinase) have recognized antiviral effects in higher animals and are believed to have antiviral effects in the transgenic plants of the present invention. PKR is stimulated by dsRNA to phosphorylate translation factor eIF2 which indirectly inhibits protein synthesis intiation. On the other hand, 2-5A synthetase is activated by dsRNA resulting in the production of "2-5A," $p_xA(2'p5'A)_y$ wherein X = about 1 to about 3 and Y ≧ about 2, from ATP. The 2-5A then activates an endoribonuclease entitled 2-5A dependent RNase (also known as RNase L or nuclease F). The activated ribonuclease degrades mRNA and rRNA thus inhibiting protein synthesis.

These above-described pathways are particularly effective at inhibiting viruses in animals with single stranded RNA genomes that replicate through dsRNA intermediates, such as the picornaviruses, and are believed to be effective at inhibiting similar types of viruses that infect plants. This belief is premised upon the understanding that most single stranded RNA plant viruses produce double stranded structures during

replication by their viral replicases, see Dawson, W.O. et al.: Acad. Press, 38:307-342 (1990), and that plant viruses are similar to animal viruses in structure, composition and mechanism of replication in cells. In addition, even viral so-called single-stranded RNA may contain secondary structures which could activate PKR and 2-5A synthetase leading to widespread plant protection against plant viruses. It is believed that co-expression of 2-5A-dependent RNase and 2-5A-synthetase, will lead to the destruction of viral mRNA and viral genomic RNA thereby protecting the transgenic plants of the present invention from viruses. Moreover, it is believed that expression of PKR by the transgenic plants of the present invention will inhibit viral protein synthesis leading to inhibition of virus replication and protection of the transgenic plants. The present invention is therefore premised in part upon the belief that plant virus RNAs activate 2-5A-synthetase and PKR in the transgenic plants of the instant invention leading to immunity against virus infection. Furthermore, expression of 2-5A synthetase alone or 2-5A-dependent RNase alone or PKR alone may protect plants against viruses, perhaps by binding to viral RNA, such as viral replicative intermediates thereby blocking viral replication. Moreover, expression of only the dsRNA binding

domains of PKR and/or of 2-5A-synthetase may similarly protect the transgenic plants of the present invention against viral infection.

It should therefore be appreciated by those versed in this art that novel transgenic plants which are resistant to viral infection can now be produced in accordance with the present invention. It is believed that the effectiveness of the anti-viral protection can be enhanced or even maximized when at least the three-above animal antiviral genes are inserted into plants to form exemplary transgenic plants of the present invention, since the animal antiviral proteins encoded by these three animal antiviral genes interfere with different stages of the viral life cycles. Moreover, these animal antiviral proteins or amino acid sequences are believed likely to be safe to give or introduce into animals, including humans, since these antiviral proteins or amino acid sequences are naturally occurring in humans as well as in other mammals, avians and reptiles.

While the present invention is described herein with reference to the particular sequences disclosed, it should nevertheless be understood by those skilled in this art that the present invention contemplates variations to the amino acid and/or nucleotide sequences which do not destroy 2-5A

synthetase activity, PKR activity and/or 2-5A-dependent ribonuclease activity. Therefore, the present invention contemplates any analogs, parts or fragments of 2-5A-dependent RNase, 2-5A synthetase, and PKR which are active, such as any active part, and any encoding sequences therefor. In other words, the present invention includes, among other things, any amino acid sequence, any nucleotide sequence and any transgenic plant which have the ability to accomplish the objectives of the instant invention. For example, the instant invention includes any amino acid sequence which has antiviral activity and any nucleotide sequence which encodes therefor and those transgenic plants that express such nucleotide sequences. More specifically, the present invention includes, for instance: 1.) any animal amino acid sequence which has the ability to inhibit or interfere with viral replication such as those amino acid sequences that have activity similar or identical to PKR activity, 2-5A synthetase activity and/or 2-5A ribonuclease activity, and any nucleotide sequence which encodes for an amino acid sequence having any such activity; and 2.) any transgenic plant having any animal antiviral nucleotide sequence which encodes any such amino acid sequence which has any such antiviral activity.

The above features and advantages of the present invention will be better understood with reference to the accompanying FIGS., Detailed Description and Examples. It should also be understood that the particular methods, amino acid sequences, encoding sequences, constructs, vectors, recombinant cells, and antiviral transgenic plants illustrating the invention are exemplary only and not to be regarded as limitations of the invention.

Brief Description of the FIGS.

Reference is now made to the accompanying FIGS. in which is shown illustrative embodiments of the present invention from which its novel features and advantages will be apparent.

FIG. 1 is the 2-5A system: a ribonuclease pathway which is believed to function in the molecular mechanism of interferon action. 5'-phosphatase, p'tase; 2'-5'-phosphodiesterase, 2'-PDE.

FIGS. 2A and 2B is a comparison of 2-5A binding activity of recombinant and naturally occurring forms of murine 2-5A-dependent RNase.

FIG. 2A is a specific affinity of truncated murine 2-5A-dependent RNase for 2-5A. UV covalent crosslinking of the ^{32}p-2-5A probe (lanes 1-7) to protein is performed after translation reactions in wheat germ extract (5 µl) with murine 2-5A-dependent

RNase mRNA (from clone ZB1) (lanes 1-3) or without added RNA (lane 4) or in extract of interferon treated mouse L cells (100 µg of protein) (lanes 5-7). Reactions are without added competitor (lanes 1, 4, and 5) or in the presence of either trimer core. $(A2'p)_2A$, (100 nM) (lanes 2 and 6) or trimer 2-5A, $p_3(A2'p)_2A$ (100 nM) (lanes 3 and 7). Lanes 8 and 9 are produced by incubating the wheat germ extract with ^{35}S-methionine in the absence or presence of 2-5A-dependent RNase mRNA, respectively.

FIG. 2B are identical chymotrypsin cleavage products and are obtained from recombinant and naturally occurring form of 2-5A-dependent RNase. Partial chymotrypsin digests (arrows) are performed on truncated 2-5A-dependent RNase (clone ZB1) produced in wheat germ extract ("Recombinant") and murine L cell 2-5A-dependent RNase ("Naturally Occurring") after crosslinking to the 2-5A probe and purification from gels.

FIGS. 3A and 3B are clonings of the complete coding sequence for human 2-5A-dependent RNase.

FIG. 3A is the construction of a human 2-5A-dependent RNase clone. The initial human 2-5A-dependent RNase cDNA clone, HZB1, is isolated from an adult human kidney cDNA library in λgt10 using radiolabeled murine 2-5A-dependent RNase cDNA

(clone ZB1) as probe. See Example. Radiolabeled HZB1 DNA is used to isolate a partially overlapping cDNA clone, HZB22, which is fused to HZB1 DNA at the NcoI site to form clone ZC1. The 5'-region of the coding sequence is obtained from a genomic SacI fragment isolated using a radiolabeled HZB22 DNA fragment as probe. Fusion of the genomic SACI fragment with ZC1 at the indicated SacI site produces clone ZC3. The coding sequence with some flanking sequences is then subcloned as a HindIII fragment into pBluescript KS(+) (Stratagene) resulting in clone ZC5. The restriction map for the composite clone, ZC5, is shown. Clone HZB1 includes nucleotides designated as 658-2223 in Table I which encode for amino acids designated as 220-741 in Table I. Clone HZB22 includes a nucleotide sequence which encodes for amino acids designated as 62-397 in Table I. Clone ZC1 includes a nucleotide sequence which encodes for amino acids designated as 62-741 in Table I. Clones ZC3 and ZC5 both include nucleotide sequences which encode for amino acids designated as 1-741 in Table I.

FIG. 3B is a nucleotide sequence and predicted amino acid sequence of human 2-5A-dependent RNase with flanking nucleotide sequences. The numbers to the right indicate the positions of nucleotides and amino acid residues.

FIG. 4 is alignment of the predicted amino acid sequences for murine and human forms of 2-5A-dependent RNase. The positions of the repeated P-loop motifs, the cysteine (Cys)-rich regions with homology to zinc fingers, and the regions of homology to protein kinase domains VI and VII are indicated. Amino acids residues which are important components of the indicated domains are represented in bold type and are italicized. Identical amino acid residues in murine and human 2-5A-dependent RNase are indicated with colon (:) symbols adjacent therebetween.

FIGS. 5A and 5B are 2-5A binding properties and ribonuclease activity of recombinant human 2-5A-dependent RNase produced in vitro.

FIG. 5A is specific affinity of recombinant human 2-5A-dependent RNase for 2-5A. Crosslinking of the 2-5A probe (lanes 1-7) to protein is performed after translation reactions in wheat germ extract (5 µl) with human 2-5A-dependent RNase mRNA (lanes 1-3) or without added RNA (lane 4) or in extract of human interferon α treated (1000 units per ml for 16 h) human HeLa cells (350 µg of protein) (lanes 5-7). Reactions were without added competitor (lanes 1, 4, and 5) or in the presence of either trimer core, $(A2'p)_2A$, (100 nM) (lanes 2 and 6) or trimer 2-5A, $p_3(A2'p)_2A$ (100 nM) (lanes 3 and 7). Incubations with ^{35}S-methionine are shown in lanes 8 to 12. Lane

8 is with wheat germ extract and human 2-5A-dependent RNase mRNA. Reticulocyte lysate preadsorbed to 2-5A-cellulose is incubated with human 2-5A-dependent RNase mRNA in the absence (lane 9) or presence (lane 10) of cycloheximide, or in the absence of added mRNA (lane 11). Lane 12 shows human 2-5A-dependent RNase which is produced in the nonadsorbed, crude reticulocyte lysate. The positions and relative molecular masses (in kDa) of the marker proteins are indicated.

FIG. 5B is reticulocyte lysate pretreated to remove endogeous 2-5A-dependent RNase and is incubated in the absence of added mRNA (■), in the presence of human 2-5A-dependent RNase mRNA without inhibitor (o, □) or in the presence of both 2-5A-dependent RNase mRNA and cycloheximide (50 µg per ml (●). See Example I. Subsequently, the recombinant 2-5A-dependent RNase (or controls) is adsorbed to 2-5A-cellulose and ribonuclease assays are performed after extensive washing of the matrix to reduce general nuclease activity. Radiolabeled substrate RNA was either poly(U) (o, ●,■) or poly(C) (□).

FIGS. 6A, 6B and 6C show levels of 2-5A-dependent RNase mRNA which are induced by interferon treatment of murine L929 cells even in the presence of cycloheximide.

FIG. 6A is a northern blot prepared with poly(A)$^+$RNA (4 µg per lane) that is isolated from murine L929 cells treated with murine interferon (α + β) (1000 units per ml) and/or cycloheximide (50 µg per ml) for different durations (indicated) which is probed with radiolabeled murine 2-5A-dependent RNase cDNA. Interferon, IFN; cycloheximide, CHI.

FIG. 6B shows levels of 2-5A-dependent RNase which are estimated from the autoradiogram shown in panel (a) with a video camera and QuickCapture and Image computer programs.

FIG. 6C shows levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as determined in the same blot shown in panel (A).

FIGS. 7A and 7B are the truncated, recombinant murine 2-5A-dependent RNase, clone ZB1, and murine L cell 2-5A-dependent RNase having identical 2-5A binding activities localized to a repeated P-loop motif.

FIG. 7A shows incubations of truncated 2-5A-dependent RNase, clone ZB1, ("Recombinant") which is produced in wheat germ extract (upper panel) or of murine L cell 2-5A-dependent RNase (labeled "Naturally Occurring," lower panel) with the ^{32}p-2-5A probe, (2.4 nM), are in the absence of presence of unlabeled 2',5'-phosphodiester linked oligonucleotides (as indicated) followed by uv covalent

crosslinking. Autoradiograms of the dried SDS/10% polyacrylamide gels are shown. Concentrations of the oligonucleotide competitors are indicated. I is inosine.

FIG. 7B shows a truncated series of murine 2-5A-dependent RNase mutants (ZB1 to ZB15) which is produced in wheat germ extract which are assayed for 2-5A binding activity by a filter binding method. See Example and Knight et al. 1980). The positions of the P-loop motifs and the lengths of the translation products are indicated. Clone ZB1 encodes for amino acids designated as 1-656 in Table 2 , except for the last 5 amino acid residues which are Lys, Pro, Leu, Ser, and Gly. Clone ZB2 encodes for amino acids designated as 1-619 in Table 2 . Clone ZB3 encodes for amino acids designated as 1-515 in Table 2 . Clone ZB5 encodes for amino acids designated as 1-474 in Table 2 . Clone ZB9 encodes for amino acids designated as 1-403 in Table 2 . Clone ZB10 encodes for amino acids designated as 1-365 in Table 2 . Clone ZB13 encodes for amino acids designated as 1-294 in Table 2 . Clone ZB14 encodes for amino acids designated as 1-265 in Table 2 . Clone ZB15 encodes for amino acids designated as 1-218 in Table 2 .

FIGS. 8A and 8B are substitution mutations of the lysine residues in the P-loop motifs of 2-5A-dependent RNase.

FIG. 8A shows the truncated murine 2-5A-dependent RNase, clone ZB1, and lysine to asparagine substitution mutants of clone ZB1, which are synthesized in wheat germ extract. In (A) unlabeled translation products are covalently crosslinked to the bromine-substituted, ^{32}P-labeled 2-5A probe, Br-2-5A-[^{32}P]pCp. See Nolan-Sorden et al., 1990.

FIG. 8B shows the mRNA species which are translated in the presence of ^{35}-S-methionine in separate reactions. Autoradiograms of the dried, SDS/polyacrylamide gels are shown. The order and positions of the translation products (labelled "RNase") and the relative molecular masses (in kDa) of the protein markers are indicated.

FIGS. 9A and 9B are a comparison of the amino acid sequences of RNase E and 2-5A-dependent RNase.

FIG. 9A shows identical and conservative matches which are shown between E. coli RNase E and the murine and human forms of 2DR.

FIG. 9B is a model for the structure and function of 2DR. Abbreviations: P-loop motifs, a repeated sequence with homology to P-loops; Cys_x, a

cysteine-rich region with homology to certain zinc fingers; PK, homology to protein kinase domains VI and VII.

FIGS. 10A and 10B are a comparison of the amino acid sequences of the ankyrin repeats in the human and murine 2-5A-dependent RNase proteins.

FIG. 10A shows murine and human forms of 2-5A-dependent RNases containing four ankyrin repeats. Homology between the ankyrin consensus sequence and the murine and human forms of 2-5A-dependent RNase are indicated. ψ, hydrophobic amino acids.

FIG. 10B is a model showing the relative positions of the four ankyrin repeats in 2-5A-dependent RNase in comparison to the position of the proposed 2-5A binding domain (↑) (the repeated P-loop motif); Cys_X, the cysteine-rich region; PK, the protein kinase homology region, and the carboxy-terminal region required for RNase activity.

FIG. 11 shows the role of 2-5A-dependent RNase in the anti-viral response of cells to interferon treatment. Interferon binds to specific cell surface receptors resulting in the generation of a signal which activates a set of genes in the cell nucleus. The genes for 2-5A synthetase are thus activated producing inactive, native 2-5A synthetase. Interferon treatment of the cell also

activates the 2-5A-dependent RNase gene (not shown in the FIGure). Subsequently, the interferon-treated cells is infected by a virus. The virus produces double stranded RNA (dsRNA) during its replicative cycle. The viral dsRNA then activates the 2-5A synthetase resulting in the production of 2-5A. The 2-5A then activates the 2-5A-dependent RNase to degrade the viral RNA thus destroying the virus itself.

FIG. 12 depicts a physical map of T: based binary vector pAM943 which is about 12 Kbp. Abbreviations: B_L, left border; B_R, right border; Kan^r, kanamycin resistance; AMT, promoter of adenyl methyl transferase gene from Chlorella virus; 35S, promoter for 35S RNA from Cauliflower mosaic virus; TER, RNA termination signal; Ovi V and Ori K origins of DNA replication.

FIG. 13 depicts physical maps of portions of certain recombinant plasmid constructs containing cDNAs encoding mammalian antiviral proteins and showing the important DNA elements in between right border and left border of T-DNAs that are transferred to plant genomes. FIG. 13A depicts a certain portion of plasmid pAM943:PK68; FIG. 13B depicts a certain portion of plasmid pAM943:muPK68; FIG. 13C depicts a certain portion of plasmid pAM943:Synthetase; FIG. 13D depicts a certain portion of plasmid

pAM943:2-5A-dep. RNase (sense); FIG. 13D/a depicts a certain portion of plasmid pAM943:2-5A-dep. RNase and FIG. 13E depicts pAM822:2-5A dep. RNase (antisense). Abbreviations: B_L, left border; B_R, right border; Kan^r, kanamycin resistance; $Hygro^r$, hygromycin resistance; AMT, promoter of adenyl methyl transferase gene from Chlorella virus; 35S, promoter for 35S RNA from Cauliflower mosaic virus; PKR, cDNA to human PKR; muPKR, cDNA to a lysine (amino acid # 296) to arginine mutant form of PKR; Synthetase, cDNA to a low molecular weight form of human 2-5A-synthetase; 2-5Adep. RNase, cDNA to human 2-5A-dependent RNase; TER, RNA termination signal.

FIG. 14 shows a physical map of Ti based binary vector pAM822 which is about 14.6 Kbp. Abbreviations: B_L, left border; B_R, right border; Kan^r, kanamycin resistance; $Hygro^r$, hygromycin resistance; Tet^r, tetracycline resistance; AMT, promoter of adenyl methyl transferase gene from Chlorella virus; 35S, promoter for 35S RNA from Cauliflower mosaic virus; TER, RNA termination signal; Ovi V, origin of DNA replication.

FIG. 15 shows expression of human 2-5A-synthetase cDNA intransgenic tobacco plants as determined by measuring mRNA levels in a Northern blot. Construct C (pAM943:Synthetase) was introduced into the plants. Total RNA was prepared from the

leaves of control (labeled "C") and transgenic plants using RNASTAT-60 (Tel-Test B., Inc.). Thirty µg of RNA was treated with glyoxal and separated in a 1.5% agarose gel. After electrophoresis RNA was transferred to Magnagraph (MSI) Nylon membrane and probed with human 2-5A-synthetase cDNA labeled with [α-^{32}P]dCTP by random priming. Autoradiograms were made from the dried blots.

FIG. 16 shows expression of mutant and wild type forms of human PKR cDNA in transgenic tobacco plants as determined by measuring mRNA levels in a Northern blot. Constructs A (pAM943:PK68) and B (pAM943:muPK68) encoding wild type and mutant (lysine at position 296 to arginine) forms of PKR, respectively, were introduced into the plants. Total RNA was prepared from the leaves of control (labeled "C") and transgenic plants using RNASTAT-60 (Tel-Test B., Inc.). Thirty µg of RNA was treated with glyoxal and separated in a 1.5% agarose gel. After electrophoresis RNA was transferred to Magnagraph(MSI) Nylon membrane and probed with human PKR cDNA labeled with [α-^{32}P]dCTP by random priming. Autoradiograms were made from the dried blots.

FIG. 17 shows a presence of 2-5A-dependent RNase cDNA in transgenic plants as determined on a Southern blot. Genomic DNA was isolated from leaves of transgenic plants containing construct D/a

(pAM943:2-5A-dep.RNase, antisense) using CTAB (cetyltrimethylammonium bromide) following the method of Rogers and Bendich (1988, Plant Molecular Biology Manual, A6, pp. 1-10, Kluwar Academic Pulbisher, Dordrecht). Ten µg of genomic DNA was digested with HindIII for 5 h at 37°C and fractionated in a 1% agarose gel followed by transfer to Magnagraph (nylon transfer membrane, Micron Separations, Inc.) using a capillary transfer method. The cDNA for 2-5A-dependent RNase (from plasmid pZC5) was labeled by random priming with [α-^{32}P]dCTP (3,000 Ci/mmole) using a Prime-a-gene kit from (Promega) according to the protocol supplied by the company. The labeled 2-5A-dependent RNase cDNA (Specific activity of 1.0 X 10^{9-} c.p.m. per µg DNA) was washed and an autoradiogram was made from the dried membrane. The sizes (in kilobases) and the positions of the DNA markers are indicated. The band indicated as "2-5A-dep. RNase cDNA" (see arrow) was absent in Southern blots of control plants (data not shown).

FIG. 18 depicts a coding sequence for human p68 kinase mRNA (PKR).

FIG. 19 depicts a translation product of the complete coding sequence for human p68 kinase mRNA (PKR) of FIG. 18.

FIG. 20 depicts a coding sequence for human 2-5A synthetase cDNA.

FIG. 21 depicts a translation product of the coding sequence for human 2-5A-synthetase of FIG. 20.

Detailed Description

By way of illustrating and providing a more complete appreciation of the present invention and many of the attendant advantages thereof, the following Detailed Description and Examples are given concerning the novel 2-5A-dependent RNases, encoding sequences therefor, recombinant nucleotide molecules, constructs, vectors, recombinant cells, antiviral transgenic plants and methods.

Because 2-5A-dependent RNase is very low in abundance (one five-hundred-thousandth of the total protein in mouse liver, Silverman, R.H. et al., J. Biol. Chem., 263:7336-7341 (1988)), its cloning requires the development of a sensitive screening method. Murine L929 cells are selected as the source of mRNA due to high basal levels of 2-5A-dependent RNase. A protocol to enhance 2-5A-dependent RNase mRNA levels is developed based on the observation that optimal induction of 2-5A-dependent RNase is obtained by treating cells with both interferon and cycloheximide, then with medium alone. See Example. The cDNA library is screened by an adaptation of techniques developed for cloning DNA binding proteins, Singh, H. et al., Cell, 52:415-423 (1988);

Singh H. et al., BioTechniques, 7:252-261 (1989), in which a bromine-substituted ^{32}P-labeled 2-5A analogue ("2-5A probe"), Example and Nolan-Sorden, N.L. et al., Anal. Biochem., 184:298-304 (1990), replaced a radiolabeled oligodeoxyribonucleotide. A single clone (ZB1) is thus isolated from about three million plaques. The protein expressed from the ZB1 clone, transferred from plaques to filter-lifts, shows reactivity to both the 2-5A probe and to a highly purified polyclonal antibody directed against 2-5A-dependent RNase.

To obtain recombinant protein for characterization, the cDNA is transcribed and translated in cell-free systems. See Example. 2-5A binding activity is then determined by covalently crosslinking the 2-5A probe to the protein with uv light, for example, Nolan-Sorden, N.L. et al., Anal. Biochem., 184:298-304 (1990). The recombinant 74 kDa protein produced in a wheat germ extract shows specific affinity for the 2-5A probe. See FIG. 2A, lanes 1 to 3. A core derivative of 2-5A lacking 5'-phosphoryl groups, $(A2'p)_2A$, fails to interfere with binding of the protein to the 2-5A probe whereas trimer 205A, $p_3(A2'p)_2A$, completely prevents probe binding. See FIG. 2A, lanes 2 and 3, respectively. There is no detectable 2-5A binding proteins in the wheat germ extract as shown in the incubation without

added RNA, FIG. 2A, lane 4. For comparison, a similar profile of 2-5A binding activity is obtained for the 80 kDa 2-5A-dependent RNase from murine L929 cells, incubated without added oligonucleotide or with $(A2'p)_2A$ or $p_3(A2'p)_2A$ as competitors. See FIG. 2A, lanes 5 to 7. The ^{35}S-labeled translation product is shown in FIG. 2A, lane 9. In a further comparison, covalent linkage of the 2-5A probe to the about 74 kDa protein and to murine L929 cell 2-5A-dependent RNase followed by partial digestion with chymotrypsin produces an identical pattern of six labeled peptides. See FIG. 2B. Similarly, partial digestion of the two labeled proteins with S. aureus V8 protease also produces identical patterns of labeled cleavage products. These results and the apparent molecular weight of about 74 kDa for the recombinant protein, as compared to about 80 kDa for 2-5A-dependent RNase, see FIG. 2A, suggests that the about 74 kDa protein is a truncated, or partial clone for 2-5A-dependent RNase.

To obtain the entire coding sequence for human 2-5A-dependent RNase, a composite DNA containing genomic and cDNA is constructed. See FIG. 3A. The initial cDNA portion of the human 2-5A-dependent RNase clone (HZB1) is obtained by screening a human kidney cDNA library with radiolabeled murine 2-5A-dependent RNase cDNA. See

Example. A genomic clone, containing the 5'-part of the coding sequence, is isolated with radiolabeled human 2-5A-dependent RNase cDNA. The nucleotide and predicted amino acid sequences of human 2-5A-dependent RNase are determined, FIG. 3B, resulting an open reading frame encoding a protein of 83,539 Da.

A comparison is made between the predicted amino acid sequences of the human and murine forms of 2-5A-dependent RNase in order to identify and evaluate the conserved regions of the proteins. See FIG. 4. The murine cDNA, clone ZB1, contains about 88% of the coding sequence for 2-5A-dependent RNase to which an additional twenty-eight 3'-codons are added from a murine genomic clone. Alignment of the murine and human forms of 2-5A-dependent RNase indicates about 65% identity between the overlapping regions. See FIG. 4. In addition, there is 73% identity between the corresponding nucleotide sequences for murine and human 2-5A-dependent RNase. The apparent translation start codons for both the murine and human 2-5A-dependent RNases, are in an appropriate context for translational initiation, namely ACCATGG and GTCATGG, respectively. See FIG. 3B. See also, for example, Kozak, M., Cell, 44:283-292 (1986). In addition, both the human and murine 2-5A-dependent RNase sequences contain

in-frame stop codons upstream of the translation start sites. See FIG. 3B.

The 2-5A binding properties of the recombinant and naturally occurring forms of human 2-5A-dependent RNase are compared by uv covalent crosslinking to the 2-5A probe. The recombinant human 2-5A-dependent RNase produces in wheat germ extract shows specific affinity for 2-5A. See FIG. 5A, lanes 1 to 3. Radiolabeling of the cloned human 2-5A-dependent RNase with the 2-5A probe is not prevented by $(A2'p)_2A$. See FIG. 5A, lanes 1 and 2. In contrast, addition of trimer 2-5A, $p_3(A2'p)_2A$, effectively competes with the 2-5A probe for binding to the recombinant 2-5A-dependent RNase. See lane 3. The same pattern of 2-5A binding activity is obtained with 2-5A-dependent RNase in an extract of interferon-treated human HeLa cells. See FIG. 5A, lanes 5 to 7. The apparent molecular weights of HeLa cell 2-5A-dependent RNase and ^{35}S-labeled recombinant human 2-5A-dependent RNase produced in reticulocyte lysate are believed to be exactly the same (about 80 kDa). See FIG. 5A, lanes 5 and 9. The recombinant human 2-5A-dependent RNase produced in wheat germ extract migrates slightly faster probably due to post-translational modifications. See FIG. 5A, lanes 1, 2 and 8.

To demonstrate and characterize the ribonuclease activity of the cloned 2-5A-dependent RNase, translation is performed in a reticulocyte lysate instead of a wheat germ extract due to the substantially greater efficiency of protein synthesis in the former system. See FIG. 5A, compare lanes 9 and 8. Prior to translation, endogenous reticulocyte 2-5A-dependent RNase is removed by adsorbing the lysate to the affinity matrix, 2-5A-cellulose. See Example. See also, Silverman, R.H., Anal. Biochem., 144:450-460 (1985). The treatment with 2-5A-cellulose effectively removes all measurable endogenous 2-5A-dependent RNase activity from the lysate, as determined by 2-5A-dependent ribonuclease assays, and FIG. 5B. In addition, the adsorption-depletion protocol did not reduce translational efficiency. FIG. 5A, lanes 9 and 12 show the ^{35}S-translation products produced in the 2-5A-cellulose-pretreated and untreated lysates, respectively.

Ribonuclease assays with recombinant 2-5A-dependent RNase are performed after immobilizing and purifying the translation product on the activating affinity matrix, 2-5A-cellulose. It was previously shown that murine L cell 2-5A-dependent RNase bound to 2-5A-cellulose, resulting in ribonuclease activity against poly(U) but not

poly(C). See Silverman, R.H., Anal. Biochem., 144:450-460 (1985). Furthermore, by washing 2-5A-dependent RNase:2-5A-cellulose prior to adding the substrate the level of general, non-2-5A-dependent RNase, is greatly reduced. See Silverman, R.H., Anal. Biochem., 144:450-460 (1985). Incubations of lysate in the absence of added mRNA or in the presence of both human 2-5A-dependent RNase mRNA and cycloheximide resulted in only low levels of poly(U) breakdown. See FIG. 5B. In addition, it is shown that cycloheximide completely prevented 2-5A-dependent RNase synthesis. See FIG. 5A, lane 10. In contrast, translation of the human 2-5A-dependent RNase mRNA, in the absence of inhibitor, results in substantial ribonuclease activity against poly(U) but not against poly(C). See FIG. 5B. The poly(U) is degraded with a half-life of about 10 minutes whereas only 20% of the poly(C) is degraded after one hour of incubation. Binding of recombinant 2-5A-dependent RNase to the affinity matrix was also shown by monitoring the presence of the ^{35}S-labeled translation product. These results are believed to demonstrate that the recombinant human 2-5A-dependent RNase produced in vitro is a functional and potent ribonuclease. Furthermore, both recombinant and naturally occurring forms of 2-5A-dependent RNase are capable of cleaving

poly(U) but not poly(C). See FIG. 5B. See also Silverman, R.H., Anal. Biochem., 144:450-460 (1985) and Floyd-Smith, G. et al., Science, 212:1020-1032 (1981).

To determine if 2-5A-dependent RNase mRNA levels are regulated by interferon, a northern blot from murine L929 cells treated with interferon and cycloheximide is probed with the radiolabeled murine 2-5A-dependent RNase cDNA. See FIG. 6. 2-5A-dependent RNase mRNA levels are enhanced three-fold by interferon (α + β) treatment even in the presence of cycloheximide. See FIGS. 6A and B, compare lanes 1 and 2). Regulation of 2-5A-dependent RNase mRNA levels by interferon as a function of time is demonstrated (FIGS. 6A and B, lanes 3 to 6. Maximum 2-5A-dependent RNase mRNA levels are observed after 14 hours of interferon treatment. See FIGS. 6A and B, lane 6. A similar increase in levels of 2-5A-dependent RNase per se is observed after interferon treatment of the cells. Relatively invariant levels of GAPDH mRNA indicates that equivalent levels of RNA are present in every lane of the blot. See FIG. 6C. These results are believed to show that the induction of 2-5A-dependent RNase expression is a primary response to interferon treatment. The murine and human 2-5A-dependent RNase mRNAs are determined from northern blots to be 5.7 kb

and 5.0 kb in length, respectively. See FIG. 6A. The 2-5A-dependent RNase coding sequences, therefore, comprise only about 40% the nucleotide sequences contained in the mRNAs.

The 2-5A binding functions of the recombinant and naturally occurring forms of murine 2-5A-dependent RNase are characterized by covalent crosslinking to the 2-5A probe in the presence of unlabeled 2-5A or 2-5A analogues as competitors. See FIG. 7A. Interestingly, although the about 74 kDa truncated 2-5A-dependent RNase is missing about 84 amino acids from its carboxy-terminus, see FIG. 4, it nonetheless possesses a 2-5A binding activity indistinguishable from that of naturally occurring 2-5A-dependent RNase. See FIG. 7A. Trimer 2-5A[$p_3(A2'p)_2A$], at about 20 nM effectively prevents the 2-5A probe from binding to either protein. See FIG. 7A, lane 8. In comparison, a 500-fold higher concentration of $(A2'p)_2A$ (10 μM) is required to prevent probe binding to both proteins. See lane 13. The dimer species, $p_3A2'pA$, is unable to prevent the 2-5A probe from binding to the proteins even at a concentration of 10μM (lane 18). However, the inosine analogue, $p_3I2'pA2'pA$, Imai, J. et al., J. Biol. Chem., 260:1390-1393 (1985), is able to prevent probe binding to both proteins but only when added at a concentration of about 1.0 μM (lane 22).

To further define sequences involved in 2-5A binding, nested 3'-deletions of the murine 2-5A-dependent RNase cDNA, clone ZB1, are constructed, transcribed in vitro, and expressed in a wheat germ extract. See FIG. 7B. The different deletion clones produces comparable amounts of polypeptide as monitored by incorporation of ^{35}S-methionine. The levels of 2-5A binding activity are determined with the 2-5A probe in both a filter binding assay, Knight, M. et al., Nature, 288:189-192 (1980), and the uv crosslinking assay, Nolan-Sorden, N.L. et al., Anal. Biochem., 184:298-304 (1990), with similar results. See FIG. 7B. Expression of clone ZB11, encoding amino acid residues 1 to 342, results in a loss of only about 26% of the 2-5A binding activity as compared to clone ZB1 (amino acids 1 to 656). See FIG. 7B. Clones intermediate in length between ZB1 and ZB11 all result in significant levels of 2-5A binding activity. In contrast, protein produced from ZB13 (amino acids 1 to 294) results in only about 38.3% of the 2-5A binding activity of clone ZB1, suggesting that a region important for the 2-5A binding function is affected. Indeed, clone ZB14 produced a protein encoding amino acids 1 to 265 which is nearly inactive in the 2-5A binding assay (only 1.9% of th activity of clone ZB1). Interestingly, the significant decrease in 2-5A

binding activity observed with ZB14 occurs with the deletion of one of two P-loop motifs; nucleotide binding domains in many proteins. See FIGS. 4 and 7B. See also Saraste, M. et al., TIBS, 14:430-434 (1990). Deletion of both P-loop motifs in clone ZB15 results in protein (amino acids 1 to 218) which is completely lacking in 2-5A binding activity. See FIG. 7B.

To probe the involvement of the consensus lysine residues in the P-loop motifs in 2-5A binding activity, site-directed mutagenesis is performed on the truncated form of murine 2-5A-dependent RNase encoded by clone ZB1. Previously, it is reported that substitution mutations of the conserved lysine residues in P-loop motifs of eucaryotic initiation factor 4A and for Bacillus anthracis adenylyl cyclase results in a loss of ATP binding and catalytic activities, respectively. See Rozen et al., Mol. Cell. Biol., 9:4061-4063 (1989) and Xia, Z. and Storm, D.R., J. Biol. Chem., 265:6517-6520 (1990). In the former study the invariant lysine residue is mutated to asparagine. See Rozen et al., Mol. Cell. Biol., 9:4061-4063 (1989). We substituted, individually and together, the consensus lysines with asparagines at positions 240 and 274 in the two P-loop motifs of 2-5A-dependent RNase. See FIG. 8 and the Example. Analysis of the effects of these

mutations on 2-5A binding activity is determined by covalently crosslinking the ^{32}P-2-5A probe to the in vitro translation products under uv light. See FIG. 8A. See also Nolan-Sorden, N.L. et al., Anal. Biochem., 184:298-304 (1990). Similar levels of proteins are synthesized from the different mRNA species as shown in separate reactions containing ^{35}S-methionine. See FIG. 8B. The three mutant forms of 2-5A-dependent RNase shows reduced binding to the 2-5A probe. See FIG. 8A, lanes 2 to 4. Clone ZB1(Lys^{240}->Asn), FIG. 8A, lane 2, expresses a mutant 2-5A-dependent RNase with a substantially reduced affinity for 2-5A; about 48.4% of the activity of clone ZB1 as determined by phosphorimager analysis (Molecular Dynamics) of the dried gel. A more modest reduction in 2-5A binding activity, to 79% of the control value, is obtained from clone ZB1(Lys^{274}->Asn). See FIG. 8A, lane 3. In contrast, 2-5A binding activity from clone ZB1(Lys^{240,274}->Asn), FIG. 8A, lane 4, in which both conserved lysine residues are replaced with asparagine residues, is reduced to only 12.2% of the activity of clone ZB1 (averaged from three separate experiments). These results suggest that the lysine residues at positions 240 and 274 function within the context of a repeated P-loop motif in the binding of 2-5A to 2-5A-dependent RNase.

The molecular cloning and expression of 2-5A-dependent RNase, the terminal factor in the 2-5A system and a key enzyme in the molecular mechanisms of interferon action is described. See FIG. 1. The recombinant proteins produced in vitro are demonstrated to possess 2-5A binding properties identical to naturally occurring forms of murine and human 2-5A-dependent RNase. See FIGS. 2, 5A, and 7. In addition, linkage of a ^{32}p-2-5A analogue to a truncated murine 2-5A-dependent RNase and to murine L cell 2-5A-dependent RNase followed by partial proteolysis reveals identical patterns of labeled peptides. See FIG. 2B. Furthermore, the full-length recombinant human 2-5A-dependent RNase isolated on the activating, affinity matrix, 2-5A-cellulose, shows potent ribonuclease activity towards poly(U) but none against poly(C). See FIG. 5B. Similarly, it is previously demonstrated that murine L cell 2-5A-dependent RNase was activated by 2-5A-cellulose resulting in the cleavage of poly(U), but not of poly(C). See Silverman, R.H., Anal. Biochem., 144:450-460 (1985). The full-length human 2-5A-dependent RNase, which is produced in reticulocyte lysate, had the same apparent molecular weight as did naturally occurring 2-5A-dependent RNase. See FIG. 5A. However, the actual molecular mass of human 2-5A-dependent RNase is determined from

the predicted amino acid sequence, FIG. 3B, to be about 83,539 Da.

Previously, it was reported that interferon enhances levels of 2-5A-dependent RNase by between two- to twenty-fold depending on the cell type. See Silverman, R.H. et al., Eur. J. Biochem., 126:333-341 (1982b) and Jacobsen, H. et al., Virology, 125:496-501 (1983a). Results presented herein suggest that the gene for 2-5A-dependent RNase may be an interferon-stimulated gene. See FIG. 6. Levels of 2-5A-dependent RNase mRNA in murine L929 cells are elevated as a function of time of interferon ($\alpha + \beta$) treatment by a factor of about three. Furthermore, the induction appeared to be a primary response to interferon treatment because it is observed in the presence of cycloheximide. Therefore, interferon is believed to regulate the 2-5A pathway by elevating levels of both 2-5A synthetases, Hovanessian, A.G. et al., Nature, 268:537-539 (1977), and 2-5A-dependent RNase, Jacobsen, H. et al., Virology, 125:496-501 (1983a). See. FIGS. 1, 6 and 11.

The cloning of 2-5A-dependent RNase reveals several features of the protein. The 2-5A binding domain is of particular interest because it is the ability of 2-5A-dependent RNase to be activated by 2-5A that sets it apart from other nucleases. By expressing nested 3'-deletions of murine

2-5A-dependent RNase, a region between amino acids residues 218 and 294 which is believed to be critical for 2-5A binding activity is identified. See FIG. 7B. Interestingly, the identified region contains a repeated P-loop motif, one from residues 229 to 241 and another from residues 253 to 275. See FIG. 4 and Table 2. When the latter P-loop motif (amino acids 253-275) is partially deleted, there is a precipitous decline in 2-5A binding activity. See clone ZB14 in FIG. 7B.

The homology with P-loops is believed to be highly conserved between the human and murine forms of 2-5A-dependent RNase; thus underscoring the belief of the importance of this region for 2-5A binding activity. See FIG. 4. The similarity to P-loops consists of the tripeptides, glycine-lysine-threonine, preceded by glycine-rich sequences. In this regard, the unusual feature of 2-5A-dependent RNase is that the P-loop motif is repeated and are in the same orientation. Adenylyl cyclase from Bacillus anthracis also contains a duplicated P-loop motif, however, the two sequences are in opposite orientation and are overlapping. See Xia, Z. and Storm, D.R., J. Biol. Chem., 265:6517-6520 (1990).

The relative importance of the conserved P-loop lysines (at positions 240 and 274) are evaluated by site-directed mutagenesis of the murine

2-5A-dependent RNase, clone ZB1. Although individual substitution mutations of the two lysines significantly reduced 2-5A binding activity, replacing both of the lysines with asparagine residues in the same mutant RNase severely represses 2-5A binding. See FIG. 8. Perhaps the trimer 2-5A requirement for activation of most forms of 2-5A-dependent RNase could be explained if the first and third adenylyl residues of 2-5A interact with the separate P-loop sequences inducing conformational changes in 2-5A-dependent RNase. In this regard, dimer 2-5A neither binds 2-5A-dependent RNase efficiently nor does it activate 2-5A-dependent RNase, FIG. 7A; Kerr, I.M. and Brown, R.E., Prod. Natl. Acad. Sci. U.S.A., 75:265-260 (1978) and Knight, M. et al., Nature, 288:189-192 (1980), perhaps because it is too short to span the two P-loop motifs. Alternately, the residual 2-5A binding activity observed in the point mutants, ZB1(Lys^{240}-)Asn) and ZB1(Lys^{274}-)Asn), and the very low affinity of the double mutant, ZB1(Lys^{240,274}-)Asn) for 2-5A, could indicate that the two P-loop motifs are parts of separate 2-5A binding domains.

Homology with protein kinase domains VI and VII is also identified in 2-5A-dependent RNase. See FIG. 4. See also Hanks, S.K. et al., Science,

241:42-52 (1988). Although domain VI is believed to be involved in ATP binding, this region in 2-5A-dependent RNase is believed not to be important for 2-5A binding because its deletion caused only a minimal reduction in affinity for 2-5A. See FIG. 7B. However, a modest (two-fold) stimulatory effect of ATP on 2-5A-dependent RNase activity has been reported. See Wreschner, D.H. et al., Eur. J. Biochem., 124:261-268 (1982) and Krause, D. et al., J. Biol. Chem., 261:6836-6839 (1986). The latter report indicated that ATP was not required for 2-5A-dependent RNase activity but may act to stabilize the enzyme. Therefore, the region of homology with protein kinases could perhaps bind ATP resulting in stimulation of ribonuclease activity through stabilization of the enzyme.

A consensus zinc finger domain, reviewed in Evans, R.M. and Hollenberg, S.M., Cell, 52:1-3 (1988), consisting of six cysteine residues with the structure $CX_4CX_3CX_{17}CX_3CX_3C$ (amino acid residues 401-436 in Table 2) is identified in the murine form of 2-5A-dependent RNase. See FIG. 4. The homologous region in the human form of 2-5A-depenent RNase is $CX_{11}CX_{25}CX_3CX_6C$ (amino acid numbers 395 to 444 in Table 1). Because zinc fingers are nucleic acid binding domains, the cysteine-rich region in 2-5A-dependent RNase could be involved in binding to

the RNA substrate. Alternatively, the cysteine-rich domain in 2-5A-dependent RNase could mediate formation of 2-5A-dependent RNase dimers. Analysis of crude preparations of 2-5A-dependent RNase suggest that 2-5A-dependent RNase may form dimers in concentrated but not in dilute extracts. See Slattery, E. et al., Proc. Natl. Acad. Sci. U.S.A., 76:4778-4782 (1979) and Wreschner, D.H. et al., Eur. J. Biochem., 124:261-268 (1982).

Comparison between the amino acid sequences of other ribonucleases with 2-5A-dependent RNase identifies some limited homology with RNase E, an endoribonuclease from E. coli. See FIG. 9A. See also Apirion D. and Lassar, A.B., J. Biol. Chem., 253:1738-1742 (1978) and Claverie-Martin, F. et al., J. Biol. Chem. 266:2843-2851 (1991). The homology with RNase E is relatively conserved between the human and murine forms of 2-5A-dependent RNase and spans a region of about 200 amino acid residues. Within these regions there are 24 and 32% identical plus conservative matches, with some gaps, between RNase E and the human and murine forms of 2-5A-dependent RNase, respectively. See FIG. 9A. The rne gene which encodes RNase E and the altered mRNA stability (ams) gene, Ono, M. and Kumano, M., J. Mol. Biol., 129:343-357 (1979), map to the same genetic locus. See Mudd E.A. et al., Mol.

Microbiol., 4:2127-2135 (1990); Babitzke, P. and Kushner, S.R., Proc. Natl. Acad. Sci. U.S.A., 88:1-5 (1991) and Taraseviciene, L. et al., Mol. Microbiol., 5:851-855 (1991). RNase E is required for both efficient mRNA turnover and rRNA processing in E. coli. See Mudd E.A. et al., Mol. Microbiol., 4:2127-2135 (1990) and Babitzke, P. and Kushner, S.R., Proc. Natl. Acad. Sci. U.S.A., 88:1-5 (1991). The cleavage specificities of 2-5A-dependent RNase and RNase E are similar in that 2-5A-dependent RNase cleaves mainly after UU or UA, Wreschner, D.H. et al., Nature, 289:414-417 (1981a) and Floyd-Smith, G. et al., Science, 212:1020-1032 (1981), and RNase E usually cleaves within the central AUU sequence of (G or A)AUU(A or U), Ehretsmann, C.P. et al., Genes & Development, 6:149-159 (1992). The location of the RNase E homology and other identified features in 2-5A-dependent RNase are shown. See FIG. 9B. These findings raise the possibility that RNase E may be the ancestral precursor of 2-5A-dependent RNase. In this regard, there are indications of 2',5'-oligoadenylates in E. coli. See Brown, R.E. and Kerr, I.M., Process in Clinical and Biological Research, 202:3-10 (1985) and Trujillo, M.A. et al., Eur. J. Biochem., 169:167-173 (1987). However, the evolutionary distribution of a complete 2-5A system (i.e. 2-5A synthetase and 2-5A-dependent RNase) is

reported to begin only with reptiles or possibly amphibia. See Cayley, P.J. et al., Biochem. Biophys. Res. Commun., 108:1243-1250 (1982).

Endoribonucleases play a controlling role in RNA metabolism by catalyzing the rate-limiting steps in RNA decay. See Brawerman, G., Cell, 57:9-10 (1989). 2-5A-dependent RNase is a uniquely regulated endoribonuclease which mediates effects of interferon against picornaviruses. It functions by binding 2-5A and subsequently degrades both viral and cellular RNA. See Wreschner, D.H. et al., Nucleic Acids Res., 9:1571-1581 (1981b). In addition, the 2-5A system may be involved in the antiproliferative effects of interferon and in the fundamental control of RNA stability. Cellular levels of 2-5A-dependent RNase and/or 2-5A-synthetase are regulated during interferon-treatment, Hovanessian, A.G. et al., Nature, 268:537-539 (1977) and Jacobsen, H. et al., Virology, 125:496-501 (1983a), cell growth arrest, Stark, G. et al., Nature, 278:471-473 (1979) and Jacobsen, H. et al., Proc. Natl. Acad. Sci. U.S.A., 80:4954-4958 (1983b), cell differentiation, Krause, D. et al., Eur. J. Biochem., 146:611-618 (1985), changing hormone status, e.g., Stark, G. et al., Nature, 278:471-473 (1979), and liver regeneration, Etienne-Smekens, M. et al., Proc. Natl. Acad. Sci. U.S.A., 80:4609-4613 (1983). However, basal levels

of 2-5A-dependent RNase and 2-5A synthetase are present in most if not all mammalian cells. The existence of multiple forms of 2-5A synthetase with different intracellular locations, Hovanessian, A.G. et al., EMBO J., 6:1273-1280 (1987), could indicate diverse functions for the 2-5A system. Similarly, the ubiquitous presence of the 2-5A system in higher animals suggests an important function for 2-5A-dependent RNase, Cayley, P.J. et al., Biochem. Biophys. Res. Commun., 108:1243-1250 (1982). For instance, 2-5A-dependent RNase cleaves rRNA at specific sites in intact ribosomes, Wreschner, D.H. et al., Nucleic Acids Res., 9:1571-1581 (1981b) and Silverman, R.H. et al., J. Virol., 46:1051-1055 (1983), possibly affecting translation rates. The transient nature of 2-5A, Williams, B.R.G. et al., Eur. J. Biochem., 92:455-562 (1978), and its growth inhibitory effect after introduction into cells, Hovanessian, A.G. and Wood, J.N., Virology, 101:81-89 (1980), indicate that the 2-5A system is a tightly regulated pathway.

EXAMPLE I

The source of mRNA for preparing the cDNA library is murine L929 cells grown in EMEM (Whittaker, Inc.) and supplemented with about 10% FBS (Gibco-BRL), and antibiotics. The cells are treated with about 50 µg per ml of cycloheximide and 1000

units per ml of murine interferon (α + β) (1.3 X 10^7 units per mg protein: Lee Biomolecular) for about 2.5 hours to increase levels of 2-5A-dependent RNase mRNA. Total RNA was then isolated, e.g. Chomczynski, P. and Sacchi, N., Anal. Biochem., 162:156-159 (1987), from which poly(A)$^+$ RNA is prepared by oligo(dT)-cellulose chromatography as described. See Sambrook, J. et al., Cold Spring Harbor Laboratory Press (1989). Synthesis of the first strand of cDNA is done by using reverse transcriptase as described (Superscript; BRL) except that 5-methyl-dCTP is substituted for dCTP and an XhoI-oligo-dT adapter-primer (Stratagene) is used. Synthesis of the second strand of cDNA and ligation of EcoRI linker was as described (Stratagene). The cDNA is digested with EcoRI and XhoI and unidirectionally cloned into predigested λZAPII vector (Stratagene). The library is packaged by using Giagpack Gold extract and titered on PLK-F bacteria.

The cDNA library is screened directly without prior amplification at a density of about 25,000 phage per 150 mm plate. Phage are grown for 3.5 hours at about 42°C until plaques are visible. Nitrocellulose filters saturated in IPTG (10 mM) and then dried, are overlaid on the plates and growth was continued for an additional 4 to 6 hours at 37°C. The filters are processed by a modification of the

methods of Singh, H. et al., Cell, 52:415-423 (1988) and Singh, H. et al., BioTechniques, 7:252-261 (1989). Filters are washed in ice-cold binding buffer (about 20 mM Tris-HCl, about pH 7.5, about 20 mM magnesium acetate, about 50 mM potassium chloride, about 1 mM EDTA, about 50 mM β-mercaptoethanol, about 0.1 mM PMSF, about 5% glycerol) containing about 6 M guanidine-HCl for about 20 min. The solution containing the filters is then diluted two-fold with binding buffer and washing on ice is continued for about an additional 5 minutes; serial two-fold dilutions were continued until the guanidine concentration was about 187 mM. The filters are then washed twice with binding buffer, and incubated with binding buffer containing about 5% nonfat milk for one hour at about room temperature. The filters are then washed twice with binding buffer and incubated in binding buffer (supplemented with about 0.25% nonfat dry milk and about 0.02% sodium azide) containing $p(A2'p)_2(br^8A2'p)_2A3'$-[32P]Cp (the "2-5A probe"), Nolan-Sorden, N.L. et al., Anal. Biochem., 184:298-304 (1990), at about 2 X 10^5 counts per minute per ml (about 3,000 Ci per mmole) at about 4°C with shaking for about 24 hours. The filters are washed twice with binding buffer and then twice with water before air drying and exposing to film.

Murine L929 cells are treated with about 1000 units per ml interferon (α + β) with or without about 50 µg per ml of cycloheximide and the total RNA is then isolated as described. See Chomczynski, P. and Sacchi N., Anal. Biochem., 162:156-159 (1987). Poly(A)$^+$ RNA is prepared by oligo(dT)-cellulose chromatography, as described in Sambrook, J. et al., Cold Spring Harbor Laboratory Press (1989), and is separated on glyoxal agarose gels and transferred to Nytran membranes. RNA is immobilized on the membrane by uv crosslinking (Stratalinker, Stratagene). The murine 2-5A-dependent RNase cDNA is ^{32}P-labeled by random priming and then hybridized to the filter [about 50% formamide, about 10% dextran sulphate, Denhardt's solution about 1% SDS, 6X SSPE, Sambrook, J. et al., Cold Spring Harbor Laboratory Press (1989), about 250 µg per ml salmon sperm DNA] at about 42°C.

The Human 2-5A-dependent RNase cDNA clone, HZB1, is isolated from an adult human kidney cDNA library in λgt10 with radiolabeled (random primed) murine 2-5A-dependent RNase cDNA (clone ZB1) as probe, Sambrook, J. et al., Cold Spring Harbor Laboratory Press (1989). Clone HBZ22 is isolated using radiolabeled HZB1 DNA as probe. The genomic human 2-5A-dependent RNase clone is isolated from a human placenta cosmid library in vector pVE15

(Stratagene) with a radiolabeled fragment of HZB22 DNA as probe. The murine genomic 2-5A-dependent RNase clone is isolated from a mouse 129SV genomic library in vector λFIXII (Stratagene) with a radiolabeled fragment of 2-5A-BP cDNA (clone ZB1) as probe. Subcloning of DNA is in Bluescript vectors (Stratagene).

Transcription of plasmids with phage RNA polymerases is in the presence of mGppppG as described (Promega) except that reaction mixtures are supplemented with 15% dimethyl sulfoxide and incubations are at about 37°C for about 90 minutes. RNA is purified through Sephadex G50 spun-columns and ethanol precipitated prior to translation. Protein synthesis was performed, as described (Promega), at about 30°C for about one hour in micrococcal nuclease-pretreated rabbit reticulocyte lysate or in an extract of wheat germ at about room temperature for about one hour and then at about 40°C for about 12 hours. Translation reactions contain about 50 μM zinc sulfate. Endogenous 2-5A-dependent RNase in the reticulocyte lysated is removed by adsorption to about 30 μM of $p_2(A2'p)_3A$ covalently attached to cellulose (2-5A-cellulose), prepared as described in Wells, J.A. et al., J. Biol. Chem., 259:1363-1370 (1984) and Silverman, R.H. and Krause, D., I.R.L. Press, Oxford, England, pp. 149-193 (1987), for about

one hour on ice as described. See Silverman, R.H., Anal. Biochem., 144:450-460 (1985). The 2-5A-dependent RNase:2-5A-cellulose complex is removed by twice centrifuging at about 400 x g for about 5 minutes at about 2°C. The supernatant completely lacking in measurable levels of 2-5A-dependent RNase. See FIG. 5.

The set of nested 3'-deletions of the truncated murine 2-5A-dependent RNase cDNA, ZB1, is generated with exonuclease III/S1 nuclease digestion followed by filling-in with Klenow DNA Polymerase using the "Erase-A-Base" system (Promega).

The synthesis of the 2-5A probe, $p(A2'p)_2(br^8A2'p)_2A[32P]Cp$, and its crosslinking to 2-5A-dependent RNase is performed exactly as described. See Nolan-Sorden, N.L. et al., Anal. Biochem., 184:298-304 (1990). Briefly, the 2-5A probe, about 0.7 to 2.5 nM at 3,0009 Ci/mmole, is incubated for about one hour on ice with cell extract prepared as described, Silverman, R.H. and Krause, D., I.R.L. Press, Oxford, England, pp. 149-193 (1987), in the absence or presence of unlabeled oligonucleotide competitors. Covalent crosslinking is done under a uv lamp (308 nm) for one hour on ice and the proteins are separated on SDS/10% polyacrylamide gels. Filter assays for 2-5A binding activity using the 2-5A probe for about one hour on

ice, as described in Knight, M. et al., Nature, 288:189-192 (1980).

Protease digestions are performed on gel-purified proteins in a gel, as described by Cleveland, D.W. et al., J. Biol. Chem., 252:1102-1106 (1977).

The ribonuclease assay with 2-5A-cellulose is performed, as described by Silverman, R.H., Anal. Biochem., 144:450-460 (1985). Briefly, lysates are adsorbed to about 30 μM of 2-5A-cellulose on ice for about two hours. The matrix is then washed three times by centrifuging and resuspending in buffer A. See Silverman, R.H., Anal. Biochem., 144:450-460 (1985). The matrix is then incubated with poly(U)-[^{32}P]Cp or poly(C)-[^{32}P]Cp (both at about 16 μM in nucleotide equivalents) at about 30°C and the levels of acid-precipitable radioactive RNA are determined by filtration on glass-fiber filters.

The Sanger dideoxy sequencing method is used to determine the DNA sequences (Sequenase, United States Biomedical).

The lysines in the truncated murine 2-5A-dependent RNase, clone ZB1, at positions 240 and 274 are mutated, individually and together, to asparagine residues. Mutants ZB1(Lys^{274}-)Asn) and the double mutant, ZB1(Lys^{240,274}-)Asn), are obtained with mutant oligonucleotides after subcloning ZB1

cDNA into pALTER-1 as described (Promega). Mutant ZB1(Lys^{240}->Asn) is obtained after polymerase chain reaction amplification of a segment of ZB1 with an upstream primer containing a unique HincII site attached to the mutant sequence and a second primer downstream of a unique Bg1II site. The HincII- and BG1II-digested polymerase chain reaction product and similarly-digested clone ZB1 are then ligated. The specific mutations are: for codon 240, AAA->AAC and for codon 274, AAG->AAC. Mutants are confirmed by DNA sequencing.

EXAMPLE II

Seeds of tobacco (Nicotiana tabacum cv. Wisconsin) and Ti based binary vectors pAM943 and pAM822 were obtained from Dr. Amit Mitra, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska. The Argobacterium tumefaciens LBA4404 and the E. coli strains K802 and MM294 were purchased from Clonetech, Palo Alto, California and Stragene, LaJolla, California. The plant tissue culture medium Murashige and Skoog's ready mix (MS media) was purchased from Sigma Chemical Company, St. Louis, Missouri. The human cDNAs for PKR, the lysine → arginine mutant PKR, and 2-5A synthetase were obtained from Dr. B.R.G. Williams, Department of Cancer Biology, The Cleveland Clinic Foundation. See, for example, Meurs, E. et al.: Cell, 62:379-390

(1990); Chong, K.L. et al.: EMBO J., 11:1553-1562 (1992); Rysieki, G. et al.: J. Interferon Res., 9:649-657 (1989); Benech, P. et al.: EMBO J., 4:2249-2256 (1985); and Saunders, M.E. et al.: EMBO J., 4:1761-1768 (1985). The human cDNA for 2-5A dependent RNase, as shown in FIG. 3A, was cloned in Dr. R.H. Silverman's laboratory in the Department of Cancer Biology and is the property of The Cleveland Clinic Foundation. See, Zhou, A. et al.: Cell, 72:753-765 (1993).

The expression vector pAM943 is used to obtain Argobacterium-mediated transfer of T DNA containing the cDNAs and kanamycin resistance marker gene. The physical map of the plasmid vector pAM943 shows its elements. See FIG. 12. The plasmid pAM943 contains a dual promoter consisting of the adenyl methyl transferase (AMT) gene promoter of Chlorella virus and the wild type 35S promoter of Cauliflower mosaic virus. The vector also contains the gene for kanamycin resistance to select the transformed plants. Initially, the cDNAs are subcloned in pAM943 and amplified in E. coli strains K802 or MM294 using tetracycline resistance as the selectable marker. The Argobacterium cells are transformed with the recombinant pAM943 plasmids and selected by growth in medium containing about 5 μg/ml of tetracycline,

about 10 µg/ml of kanamycin and about 25 µg/ml of streptomycin.

To subclone cDNAs for PKR (PK68), a lysine → arginine mutant PKR (muPk68; the mutant PKR protein binds to dsRNA but has no kinase activity and will thus function as a control), and a low molecular weight form of 2-5A-synthetase (synthetase), the plasmids pKS(+)PKR, pKS(+)muPKR, and pKS(+)synthetase are digested first with XbaI and than with ClaI restriction endonucleases, the cDNA fragments are purified from low melting point agarose gels and subcloned in sense orientation at XbaI and ClaI sites of pAM943. See FIG. 13. The recombinant plasmids, e.g., construct A, pAM943:PK68, construct B, pAM943:muPK68, and contruct C, pAM943:synthetase, which correspond to the constructs depicted in FIG. 13A-C, respectively, are used to transform Argobacterium tumefaciens LBA4404. The resultant bacteria, identified as AG68, AGmu68 and AGsyn, respectively, are used for tobacco leaf disc transformations. Production of the recombinant plasmids, i.e., construct A, pAM943:PK68, construct B, pAM943:muPK68, and construct C pAM943:synthetase, is described in greater detail hereinafter.

To subclone cDNA for 2-5A-dependent RNase, the plasmid pKS(+)2C5 DNA is digested with HindIII enzyme and subcloned in the HindIII site of pAM943 in

both orientations, see FIG. 13, and the recombinant plasmids, construct D, pAM943:2-5A-dep. RNase sense and construct D/a, pAM943:2-5A-dep. RNase antisense, both of which correspond to constructs D and D/a, respectively, in FIG. 13D and D/a, are used to transform Argobacterium to obtain the bacteria called AG2DR sense and AG2DR antisense, respectively. Production of the recombinant plasmids, i.e., construct D, pAM943:2-5A-dep. RNase sense, construct D/a, pAM943:2-5A-dep. RNase antisense, and construct E, pAM822:2-5A dep. RNase antisense, is also described in greater detail hereinafter.

The competent Argobacterium cells are prepared and transformation follows the method of, for example, An, G. et al.: Plant Molecular Biology Manual, AD:1-19 (1988). The presence of recombinant plasmids in the transformed Argobacterium cells is confirmed by preparing plasmid DNA and by performing PCR using specific complementary oligonucleotides and by observing restriction enzyme digests.

The physical map of plasmid pAM822, one of the vectors used to deliver the reverse orientation cDNA for 2-5A dependent RNase into plant cells by electroporation, is also shown. See FIGS. 13E and 14. To subclone cDNA for 2-5A-dependent RNase into pAM822 the entire coding region of 2-5A-dependent RNase was PCR amplified using two oligonucleotide

primers containing BamHI restriction sites before ATG (start codon) and after TGA (stop codon). The product was digested with BamHI and subcloned at Bg1II site of pAM822 vector. The cDNA used for 2-5A-dependent RNase is in plasmid pZC5 referenced in Zhou et al. Cell 72, 753-765 (1994), the human form of the cDNA. The sequence is also disclosed herein. The plasmid pAM822 contains a second selectable marker gene, the hygromycin resistance gene, permitting the construction of plants containing both 2-5A-synthetase and 2-5A-dependent RNase cDNAs. Insertion of pAM822:2-5Adep. RNase (Fig. 13E), containing 2-5A-dependent RNase cDNA, into kanamycin-resistant, transgenic tobacco leaf discs containing 2-5A-synthetase cDNA is thus performed.

Tobacco plants are grown aseptically in Murashige and Skoog's medium, known as MS medium, containing about 3% sucrose (MSO medium) and about 0.8% agar in plastic boxes (Phytatray) at about 28°C under cycles consisting of about 16 hr of light and about 8 hr of dark in a growth chamber. Leaves bigger than about 2" long are cut into about 2 to 3 cm^2 pieces under the MSO medium and 6-8 leaf pieces are placed in a 6 cm Petri dish containing about 2 ml of MSO medium and holes are made in the leaf pieces with a sterile pointed forcep. Overnight cultures of AG68, AGmu68, AGSyn, AG2DR sense and AG2DR antisense

are grown in LB (L broth) containing about 50 μM of acetosyringone and appropriate antibiotics at about 28°C in a waterbath. One hundred microliter of overnight culture is added to each of the Petri dishes containing leaf pieces. Incubation is at about 28°C under diffuse light in the growth chamber for about 2 days. Leaf pieces are washed extensively with MSO medium and transferred to solid agar for selection in shoot regeneration medium [MSO; about 0.5 mg/l BAP (benzylaminopurine); about 200 μg/ml kanamycin; about 200 μg/ml carbenicillin; and about 100 μg/ml of cefotaxine], under diffuse light at about 28°C in the growth chamber. Within about 3 weeks, regeneration of plantlets is observed. When the plantlets are about 2-3cm long they are transferred to root-inducing, hormone-free MSO solid agar medium containing about 200 μg/ml kanamycin and about 200 μg/ml carbenicillin. The transgenic plants expressing 2-5A synthetase are substantially transformed to introduce the cDNA for 2-5A-dependent RNase (with pAM943:2-5Adep.RNase sense, construct D; FIG. 13D). Alternatively, the vector pAM822 (FIG. 14) containing the 2-5A-dependent RNase cDNA in sense orientation and the hygromycin resistance gene is used to transform 2-5A-synthetase containing plants. This allows selection in hygromycin containing MSO media. Tissue culture and regeneration of plants are

done as described above. Transgenic plants are grown to produce flowers and seeds to demonstrate the transfer of the antiviral genes or nucleotide sequences to subsequent generations. Although specific plasmid constructs are described herein, the present invention is intended to include any plant vector including those with inducible promoters.

Expression of PKR, mutant PKR, 2-5A-synthetase, and 2-5A-dependent RNase in plants that are 4" to 5" tall are tested in protein extracts of leaves (supernatant of 10,000 x g centrifugation). Results of Northern and Southern blot assays and functional binding assays for 2-5A-dependent RNase are reported in Tables I-V. See also FIG. 15 wherein expression of human 2-5A synthetase cDNA in transgenic tobacco plants as determined by measuring the mRNA levels in a Northern blot is shown. FIG. 16, on the other hand, shows expression of mutant and wild type forms of human PKR cDNA in transgenic tobacco plants as determined by measuring mRNA levels in a Northern blot. FIG. 17 depicts presence of 2-5A-dependent RNase cDNA in transgenic tobacco plants as determined on a Southern blot.

TABLE I

Transgenic Tobacco Plants Expressing
Wild Type and Mutant Forms of Human PKR cDNA

(plasmid pAM943:PK68) FIG. 13A
(plasmid pAM943:muPK68) FIG. 13B

Transgenic:	Plant: (clone #)	Southern Blot: (presence of DNA)	Northern Blot: (expression of mRNA)
Mutant PKR: (plasmid pAM943:PK68) FIG. 13A	1	+	N.T.
	2	++	+
	4	N.T.	N.T.
	6	N.T.	+
	7	N.T.	+
	10	N.T.	+
	11	N.T.	+
	12	N.T.	+
	17	N.T.	+
Wild Type PKR: (plasmid pAM943:muPK68) FIG. 13B	1	N.T.	+
	2	N.T.	N.T.
	5	N.T.	+
	6	N.T.	N.T.
	7	N.T.	N.T.
	8	N.T.	+
	10	N.T.	+
	20	N.T.	N.T.
	22	N.T.	N.T.

N.T., Not Tested

TABLE II

Transgenic Tobacco Plants Expressing
Human 2-5A-Synthetase cDNA

(Plasmid pAM943:synthetase - FIG. 13C)

Plant: (clone#)	Southern Blot: (presence of DNA)	Northern Blot: (expression of mRNA)
1	++	+
3	±	N.T.
4	+	++
5	±	N.T.
6	±	N.T.
7	±	N.T.
8	+++	+
9	+	N.T.
10	+	+
12	+	N.T.
13	+	N.T.
14	++	-
15	+	±
16	+	-
17	N.T.	++
18	N.T.	++
a	N.T.	N.T.
b	N.T.	N.T.
c	N.T.	N.T.
d	N.T.	N.T.

N.T., Not Tested.

TABLE III

Transgenic Tobacco Plants Containing Sense or Antisense Orientation Human 2-5A-Dependent RNase cDNA

(plasmid pAM943:2-5A-dep. RNase sense - FIG. 13D)
(plasmid pAM943:2-5A-dep. RNase antisense - FIG. 13D/a)

Transgenic:	Plant: (clone #)	Southern (presence of DNA)	Northern (expression of mRNA)	2-5A-Binding Assay: (protein activity
Antisense:	1	+	N.T.	N.T.
	2	+	N.T.	N.T.
	3	+	N.T.	N.T.
	4	+	N.T.	N.T.
	5	+	N.T.	N.T.
	a	N.T.	N.T.	N.T.
	b	N.T.	N.T.	N.T.
	c	N.T.	N.T.	N.T.
Sense:	Z1	+	-	+
	Z2	++	-	++
	Z3	++	N.T.	++
	Z4	+	N.T.	N.T.
	Z5	N.T.	N.T.	+++
	Z6	N.T.	N.T.	++
	Z7	N.T.	N.T.	+/-

N.T., Not Tested.

TABLE IV

Transgenic Tobacco Plants Containing Both Human 2-5A-Synthetase and Human 2-5A-Dependent RNase cDNA

(plasmid pAM943:synthetase - FIG. 13C)
(plasmid pAM943:2-5A-dep. RNase sense - FIG. 13D)

Plant: (clone #)	Southern Blots: (2-5A-Syn DNA)	(2-5A-Dep. RNase DNA)	Northern Blot: (2-5A Syn. mRNA)	(2-5A-dep. RNase mRNA
14/1	N.T.	-	+	-
14/2	N.T.	-	+	-
14/3	N.T.	N.T.	N.T.	N.T.
14/4	N.T.	N.T.	N.T.	N.T.
14/5	N.T.	N.T.	N.T.	N.T.
14/6	N.T.	N.T.	N.T.	N.T.
15/1	N.T.	-	+	-
15/2	N.T.	-	+	-
15/3	N.T.	-	+	-
15/4	N.T.	N.T.	+	-
15/5	N.T.	N.T.	N.T.	N.T.
15/6	N.T.	-	+	-
15/7	N.T.	-	N.T.	N.T.

N.T., Not Tested.

Assays of dsRNA-dependent autophosphorylation of PKR, 2-5A synthetase activated with dsRNA, and 2-5A-dependent RNase by UV-crosslinking to radioactive 2-5A, see Nolan-Sorden et al.: Analytical Biochemists, (184):298-304 (1990), may be performed on the leaf extracts. The levels of the proteins may also be determined by Western blot analysis using the antibodies against PKR, 2-5A-synthetase and 2-5A-dependent RNase.

To demonstrate the expression of 2-5A-dependent RNase in transgenic plants containing construct D, pAM943:2-5A-dep. RNase sense, as depicted in FIG. 13D, functional assays that measure binding of radiolabeled 2-5A analog to 2-5A-dependent RNase are performed. See Tables III and V. Results show the presence of 2-5A-dependent RNase in transgenic plants Z1, Z2, Z3, Z5 and Z6. It is believed that the highest levels of human, recombinant 2-5A dependent RNase are in plant Z5. See Table V.

TABLE V

Functional Expression of 2-5A-Dependent RNase in Transgenic Tobacco Plants ad Determined by a 2-5A Binding Assay

(plasmid pAM943:2-5A-dep. RNase sense - FIG. 13D)

Plant;	2-5A Binding Activity[a]:
Z1	662
Z2	1,618
Z3	1,545
Z5	2,575
Z6	1,547
Z7	31

[a]Tobacco plants contain construct D, pAM943:2-5Adep. RNase (sense). 2-5A binding assays are performed by the filter binding method of Knight, M. et al. Nature (288):189-192 (1980) with modifications. A ^{32}P-labeled and bromine substituted 2-5A analog, $p(A2'p)_2(br^8A2'p)_2A3'-^{32}p]Cp$, about 15,000 counts per min per assay, at about 3,000 Ci per mmole, Nolan-Sorden, N.L., et al. Anal. Biochem., (184):298-304 (1990), is incubated with plant extracts, containing about 100 micrograms of protein per assay, on ice for about 4 h. The reaction mixtures are then transferred to nitrocellulose filteres which are washed twice in distilled water and dried and the amount of 2-5A probe bound to the 2-5A-dependent RNase on the filters is measured by scintillation counting, Silverman, R.H. and Krause, D., In, Clemens, M.J., Morris, A.G., and Gearing. A.J.H., (eds.), Lymphokines and Interferons - A Practical Approach, I.R.L. Press, Oxford, pp. 149-193 (1987). Data is presented as counts per min of labeled 2-5A bound to 2-5A-dependent RNase expressed in the transgenic plants. Background radioactivity from extracts of control plants, 705 counts per min, consisting of nonspecific binding of 2-5A, is subtracted from these data.

To further confirm that the transgenic plants containing 2-5A-dependent RNase cDNA express functional 2-5A-dependent RNase protein or an amino acid sequence, an affinity labeling method is performed (data not shown). In this method, 2-5A-binding activity is determined on a Western blot with a bromine-substituted, ^{32}p-labeled 2-5A analog (the "probe"), as described in Nolan-Sorden, N.L. et al.: Anal. Biochem., 184:298-304 (1990). More particularly, leaves are collected from transgenic plants containing 2-5A-dependent RNase cDNA and they are homogenized in NP40 lysis buffer, see Silverman, R.H. and Krause, D. (1987) In, Clemens, M.J., Morris, A.G., and Gearing, A.J.H., (eds.), Lymphokines and Interferons - A Practical Approach, I.R.I., Press, Oxford, pp. 149-193, supplemented with about 5mM ascorbic acid, about 1 mM cysteine, about 2 µg per ml leupeptin, about 100 µ per ml phenylmethyleulfonyl fluoride, and about 2 µg per ml pepstatin. Extracts are clarified by centrifugation at about 10,000 x g for about 10 min. Supernatants of the extracts, about 100 µg of protein per assay, are separated by SDS/10% polyacrylamide gel electrophoresis, followed by transfer of the proteins to Immobilon-P membrane filters (Millipore Corp., Bedford, MA). The filter is then incubated with about 4 X 10^5 c.p.m. per ml of ^{32}p-labeled 2-5A probe for about 24 h at about 4°C,

according to Zhou, A. et al.: Cell 73:753-765 (1993). The autoradiograms of the washed and dried filters show the presence of functional human 2-5A-dependent RNase visible to about 80 kDa bands, in plants Z3, Z5, and Z6 (data not shown).

Antiviral activity of the plants are determined by rubbing celite powder coated with Tobacco mosaic virus (ATCC) and Tobacco Etch virus (from Dr. Amit Mitra, Nebraska). The plants are monitored for symptoms of viral infection on leaves from control and transgenic plants and are documented in photographs.

The plasmids described and the transformed Argobacterium strains can be used to transform any other plants into virus-resistant plants. Exemplary of plants that may be transformed in accordance with the present invention include vegetable plants like corn, potato, carrot, lettuce, cabbage, broccoli, cauliflower, bean, squash, pumpkin, pepper, onion, tomato, pea, beet, celery, cucumber, turnip and radish plants, fruit plants like banana, apple, pear, plum , apricot, peach, nectarine, cherry, key lime, orange, lemon, lime, grapefruit, grape, berry, and melon plants, grain plants like wheat, barley, rice, oat and rye plants, grass, flowers, trees, shrubs and weeds such as laboratory weeds like Arabidopsis. It should therefore be understood that the present

invention includes any plant into which any nucleotide sequence encoding an amino acid having antiviral activity has been introduced to form transgenic plants having immunity or resistance against viral infection.

Construction of pAM943:PKR (Construct A) and pAM943;MuPKR (construct B)

The plasmids pKS(+)PKR and pKS(+)muPKR, encoding wild type PKR and a lysine to arginine at codon 296 mutant form of PKR, respectively, present in E. coli cells (obtained from Dr. B.R.G. Williams, Cleveland Clinic, Cleveland, Ohio) are prepared by standard methods. See, for example, Katze, M.G. et al.: Mol. Cell Biol., 11:5497-5505 (1991) for generation of muPKR, lysine - 296 → arginine mutant (K296R), by site specific mutagenesis as described. The PKR nucleotide sequence utilized to construct plasmids pKS(+)PKR and pKS(+)muPKR is depicted in FIG. 18. To determine the ability of a plant translation apparatus to synthesize PKR protein, capped PKR mRNA is produced from linearized pKS(+)PKR by in vitro transcription. The RNA is then translated in wheat germ extract (obtained from Promega Corp., Madison, W.I.) in the presence of ^{35}S-methionine. Synthesis of the ^{35}S-labeled PKR is detected in an autoradiogram of the dried, SDS/polyacrylamide gel.

The cDNAs encoding PKR and muPKR are excised from plasmids pKS(+)PKR and pKS(+)muPKR by digesting with KpnI and XbaI. The resulting DNA fragments containing the entire coding sequences for PKR and muPKR are purified from a low melting point agarose gel. To generate cDNAs containing at the 5' end XbaI and at the 3' end ClaI sites, the PKR cDNA and muPKR cDNA are then digested with ClaI and purified. The resulting digested PKR cDNA and muPKR cDNA are then force cloned into XbaI and ClaI digested pAM943 by DNA ligation. The resulting plasmids, FIG. 13, constructs A and B, are used to transform Argobacterium tumefaciens strain LBA4404 (Clonetech, Plao Alto, CA). Recombinant plasmids are prepared from transformed Argobacterium tumefaciens bacteria by standard methods and the presence of PKR and muPKR cDNA is confirmed by PCR analysis and restriction enzyme digests of the isolated plasmids.

Construction of pAM943:Synthetase (construct C)

The plasmid ptac-15 containing the human cDNA illustrated in FIG. 20 for a small form of 2-5A-synthetase (producing a 1.8 kb mRNA) (obtained from Dr. B.R.G. Williams, Cleveland Clinic, Cleveland, Ohio) is prepared by standard methods and is digested with BamHI and EcoRI. The synthetase cDNA is purified from a low melting point agarose gel by standard methods and is then subcloned into

plasmid pKS(+) (Strategene, La Jolla, CA) in BamHI and EcoRI sites. The resulting recombinant plasmid DNA (pKS(+)synthetase) is digested with XbaI and ClaI and the 2-5A synthetase cDNA is purified from a low melting point agarose gel and is then subcloned into XbaI and ClaI digested pAM943 to produce construct C (FIG. 13). Recombinant plasmids are prepared from transformed Argobacterium tumefaciens bacteria by standard methods and the presence of 2-5A-synthetase cDNA is confirmed by PCR analysis and by restriction enzyme digests of the isolated plasmids.

Construction of pAM943:2-5Adep.RNase sense (construct D) and pAM943:2-5Adep.RNase antisense (construct D/a)

The plasmid pKS(+)ZC5 encoding a complete coding sequence for human 2-5A-dependent RNase is digested with HindIII. The 2.5kbp cDNA for 2-5A-dependent RNase is purified in a low melting point agarose gel and is then subcloned in HindIII digested pAM943 in both sense (forward) and antisense (reverse) orientations to produce pAM943:2-5Adep.RNase sense (construct D) and pAM943:2-5Adep.RNase antisense (construct D/a), as depicted in FIG. 13D and D/a, respectively. Transformed Argobacterium are determined to contain the 2-5A-dependent RNase cDNA by restriction enzyme digests and by PCR analysis.

Construction of pAM822:2-5Adep.RNase antisense (construct E)

Polymerase chain reactions (PCR) are performed on plasmid pKS(+)ZC5 encoding human 2-5A-dependent RNase to generate HindIII and BamHI sites on the two ends of the cDNA and to reduce 5' and 3' untranslated sequences. The PCR primers used are:

ID SEQ NO:7:

2DR-5 5'-TCATGCTCGAGAAGCTTGGATCCACCATGGAGAGCAGGGAT-3'; and

ID SEQ NO:8:

H2DR-4 5'-GATACTCGAGAAGCTTGCATCCTCATCAGCACCCAGGGCTGG-3'.

The PCR product (about 2.25 kbp) is purified on a low melting point agarose gel and is then digested with HindIII and is then subcloned into HindIII digested plasmid pKS(+). The resulting plasmid, pKS:pZC5 is digested with BamHI and the 2-5A-dependent RNase cDNA fragment is purified and cloned into BglII digested pAM822. Recombinants isolated in the reverse (antisense) orientation give pAM822:2-5Adep.RNase antisense (construct E). See FIG. 13E.

As to the nucleotide sequences disclosed herein, A means adenine; C means cytosine; G means guanine; T means thymine; and U means uracil. With respect to the disclosed amino acid sequences, A means ala or alanine: R means arg or arginine; N means asn or asparagine; D means asp or aspartic acid; C means cys or cysteine; E means glu or glutamic acid; Q means gln or glutamine; G means gly or glycine; H means his or histidine; I means ile or isoleucine; L means leu or leucine; K means lys or Lysine; M means met or methionine; F means phe or phenylalanine; P means pro or proline; S means ser or serine; T means thr or threonine; W means trp or tryptophan; Y means tyr or tyrosine; and V means val or valine.

The following listed materials are on deposit under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, USA, and have been assigned the following Accession Numbers.

Plasmid DNA	ATCC No.	Deposit Date	Viability Date
pAM943:PK68 (Plasmid pA)	75996	21 Dec. 1994	13 Jan. 1995
pAM943:muPK68 (Plasmid pB)	75997	21 Dec. 1994	13 Jan. 1995
pAM943:Synthetase (Plasmid pC)	75998	21 Dec. 1994	13 Jan. 1995
pAM943:2-5Adep.RNase (Plasmid pD)	75999	21 Dec. 1994	13 Jan. 1995
Z9*, expressing, human 2-5A-dependent RNase cDNA	97047	01 Feb. 1995	07 Feb. 1995
15/2** expressing human 2-5A-synthetase cDNA	97041	01 Feb. 1995	07 Feb. 1995

*this seed contains construct D, shown in Fig. 13, which is pAM943:2-5Adep.RNase
**this seed contains construct C, shown in Fig. 13, which is pAM943:Synthetase

TABLE 1

Human 2-5A-depedent RNase

SEQ ID NO:1:, SEQ ID NO:2:, SEQ ID NO:3: and SEQ ID NO:4:

```
            -103 aatcccaacttacactcaaagct
tctttgattaagtgctaggagataaatttgcattttctca
aggaaaaggctaaaagtggtagcaggtggcatttaccgtc

ATG GAG AGC AGG GAT CAT AAC AAC CCC CAG          30
Met Glu Ser Arg Asp His Asn Asn Pro Gln          10

GAG GGA CCC ACG TCC TCC AGC GGT AGA AGG          60
Glu Gly Pro Thr Ser Ser Ser Gly Arg Arg          20

GCT GCA GTG GAA GAC AAT CAC TTG CTG ATT          90
Ala Ala Val Glu Asp Asn His Leu Leu Ile          30

AAA GCT GTT CAA AAC GAA GAT GTT GAC CTG         120
Lys Ala Val Gln Asn Glu Asp Val Asp Leu          40

GTC CAG CAA TTG CTG GAA GGT GGA GCC AAT         150
Val Gln Gln Leu Leu Glu Gly Gly Ala Asn          50

GTT AAT TTC CAG GAA GAG GAA GGG GGC TGG         180
Val Asn Phe Gln Glu Glu Glu Gly Gly Trp          60

ACA CCT CTG CAT AAC GCA GTA CAA ATG AGC         210
Thr Pro Leu His Asn Ala Val Gln Met Ser          70

AGG GAG GAC ATT GTG GAA CTT CTG CTT CGT         240
Arg Glu Asp Ile Val Glu Leu Leu Leu Arg          80

CAT GGT GCT GAC CCT GTT CTG AGG AAG AAG         270
His Gly Ala Asp Pro Val Leu Arg Lys Lys          90

                (CCT)*
AAT GGG GCC ACG CTT TTT ATC CTC GCA GCG         300
Asn Gly Ala Thr Leu Phe Ile Leu Ala Ala         100
                (Pro)*

ATT GCG GGG AGC GTG AAG CTG CTG AAA CTT         330
Ile Ala Gly Ser Val Lys Leu Leu Lys Leu         110

TTC CTT TCT AAA GGA GCA GAT GTC AAT GAG         360
Phe Leu Ser Lys Gly Ala Asp Val Asn Glu         120

TGT GAT TTT TAT GGC TTC ACA GCC TTC ATG         390
Cys Asp Phe Tyr Gly Phe Thr Ala Phe Met         130

GAA GCC GCT GTG TAT GGT AAG GTC AAA GCC         420
Glu Ala Ala Val Tyr Gly Lys Val Lys Ala         140
```

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CTA AAA TTC CTT TAT AAG AGA GGA GCA AAT          450
Leu Lys Phe Leu Tyr Lys Arg Gly Ala Asn          150

GTG AAT TTG AGG CGA AAG ACA AAG GAG GAT          480
Val Asn Leu Arg Arg Lys Thr Lys Glu Asp          160

CAA GAG CGG CTG AGG AAA GGA GGG GCC ACA          510
Gln Glu Arg Leu Arg Lys Gly Gly Ala Thr          170

GCT CTC ATG GAC GCT GCT GAA AAA GGA CAC          540
Ala Leu Met Asp Ala Ala Glu Lys Gly His          180

GTA GAG GTC TTG AAG ATT CTC CTT GAT GAG          570
Val Glu Val Leu Lys Ile Leu Leu Asp Glu          190

ATG GGG GCA GAT GTA AAC GCC TGT GAC AAT          600
Met Gly Ala Asp Val Asn Ala Cys Asp Asn          200

ATG GGC AGA AAT GCC TTG ATC CAT GCT CTC          630
Met Gly Arg Asn Ala Leu Ile His Ala Leu          210

CTG AGC TCT GAC GAT AGT GAT GTG GAG GCT          660
Leu Ser Ser Asp Asp Ser Asp Val Glu Ala          220

ATT ACG CAT CTG CTG CTG GAC CAT GGG GCT          690
Ile Thr His Leu Leu Leu Asp His Gly Ala          230

GAT GTC AAT GTG AGG GGA GAA AGA GGG AAG          720
Asp Val Asn Val Arg Gly Glu Arg Gly Lys          240

ACT CCC CTG ATC CTG GCA GTG GAG AAG AAG          750
Thr Pro Leu Ile Leu Ala Val Glu Lys Lys          250

CAC TTG GGT TTG GTG CAG AGG CTT CTG GAG          780
His Leu Gly Leu Val Gln Arg Leu Leu Glu          260

CAA GAG CAC ATA GAG ATT AAT GAC ACA GAC          810
Gln Glu His Ile Glu Ile Asn Asp Thr Asp          270

AGT GAT GGC AAA ACA GCA CTG CTG CTT GCT          840
Ser Asp Gly Lys Thr Ala Leu Leu Leu Ala          280

GTT GAA CTC AAA CTG AAG AAA ATC GCC GAG          870
Val Glu Leu Lys Leu Lys Lys Ile Ala Glu          290

TTG CTG TGC AAA CGT GGA GCC AGT ACA GAT          900
Leu Leu Cys Lys Arg Gly Ala Ser Thr Asp          300

TGT GGG GAT CTT GTT ATG ACA GCG AGG CGG          930
Cys Gly Asp Leu Val Met Thr Ala Arg Arg          310

AAT TAT GAC CAT TCC CTT GTG AAG GTT CTT          960
Asn Tyr Asp His Ser Leu Val Lys Val Leu          320
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```
CTC TCT CAT GGA GCC AAA GAA GAT TTT CAC          990
Leu Ser His Gly Ala Lys Glu Asp Phe His          330

CCT CCT GCT GAA GAC TGG AAG CCT CAG AGC         1020
Pro Pro Ala Glu Asp Trp Lys Pro Gln Ser          340

TCA CAC TGG GGG GCA GCC CTG AAG GAT CTC         1050
Ser His Trp Gly Ala Ala Leu Lys Asp Leu          350

CAC AGA ATA TAC CGC CCT ATG ATT GGC AAA         1080
His Arg Ile Tyr Arg Pro Met Ile Gly Lys          360

CTC AAG TTC TTT ATT GAT GAA AAA TAC AAA         1110
Leu Lys Phe Phe Ile Asp Glu Lys Tyr Lys          370

ATT GCT GAT ACT TCA GAA GGA GGC ATC TAC         1140
Ile Ala Asp Thr Ser Glu Gly Gly Ile Tyr          380

CTG GGG TTC TAT GAG AAG CAA GAA GTA GCT         1170
Leu Gly Phe Tyr Glu Lys Gln Glu Val Ala          390

GTG AAG ACG TTC TGT GAG GGC AGC CCA CGT         1200
Val Lys Thr Phe Cys Glu Gly Ser Pro Arg          400

GCA CAG CGG GAA GTC TCT TGT CTG CAA AGC         1230
Ala Gln Arg Glu Val Ser Cys Leu Gln Ser          410

AGC CGA GAG AAC AGT CAC TTG GTG ACA TTC         1260
Ser Arg Glu Asn Ser His Leu Val Thr Phe          420

TAT GGG AGT GAG AGC CAC AGG GGC CAC TTG         1290
Tyr Gly Ser Glu Ser His Arg Gly His Leu          430

TTT GTG TGT GTC ACC CTC TGT GAG CAG ACT         1320
Phe Val Cys Val Thr Leu Cys Glu Gln Thr          440

CTG GAA GCG TGT TTG GAT GTG CAC AGA GGG         1350
Leu Glu Ala Cys Leu Asp Val His Arg Gly          450

GAA GAT GTG GAA AAT GAG GAA GAT GAA TTT         1380
Glu Asp Val Glu Asn Glu Glu Asp Glu Phe          460

GCC CGA AAT GTC CTG TCA TCT ATA TTT AAG         1410
Ala Arg Asn Val Leu Ser Ser Ile Phe Lys          470

GCT GTT CAA GAA CTA CAC TTG TCC TGT GGA         1440
Ala Val Gln Glu Leu His Leu Ser Cys Gly          480

TAC ACC CAC CAG GAT CTG CAA CCA CAA AAC         1470
Tyr Thr His Gln Asp Leu Gln Pro Gln Asn          490

ATC TTA ATA GAT TCT AAG AAA GCT GCT CAC         1500
Ile Leu Ile Asp Ser Lys Lys Ala Ala His          500
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CTG GCA GAT TTT GAT AAG AGC ATC AAG TGG          1530
Leu Ala Asp Phe Asp Lys Ser Ile Lys Trp           510

GCT GGA GAT CCA CAG GAA GTC AAG AGA GAT          1560
Ala Gly Asp Pro Gln Glu Val Lys Arg Asp           520

CTA GAG GAC CTT GGA CGG CTG GTC CTC TAT          1590
Leu Glu Asp Leu Gly Arg Leu Val Leu Tyr           530

GTG GTA AAG AAG GGA AGC ATC TCA TTT GAG          1620
Val Val Lys Lys Gly Ser Ile Ser Phe Glu           540

GAT CTG AAA GCT CAA AGT AAT GAA GAG GTG          1650
Asp Leu Lys Ala Gln Ser Asn Glu Glu Val           550

GTT CAA CTT TCT CCA GAT GAG GAA ACT AAG          1680
Val Gln Leu Ser Pro Asp Glu Glu Thr Lys           560

GAC CTC ATT CAT CGT CTC TTC CAT CCT GGG          1710
Asp Leu Ile His Arg Leu Phe His Pro Gly           570

GAA CAT GTG AGG GAC TGT CTG AGT GAC CTG          1740
Glu His Val Arg Asp Cys Leu Ser Asp Leu           580

CTG GGT CAT CCC TTC TTT TGG ACT TGG GAG          1770
Leu Gly His Pro Phe Phe Trp Thr Trp Glu           590

AGC CGC TAT AGG ACG CTT CGG AAT GTG GGA          1800
Ser Arg Tyr Arg Thr Leu Arg Asn Val Gly           600

AAT GAA TCC GAC ATC AAA ACA CGA AAA TCT          1830
Asn Glu Ser Asp Ile Lys Thr Arg Lys Ser           610

GAA AGT GAG ATC CTC AGA CTA CTG CAA CCT          1860
Glu Ser Glu Ile Leu Arg Leu Leu Gln Pro           620

GGG CCT TCT GAA CAT TCC AAA AGT TTT GAC          1890
Gly Pro Ser Glu His Ser Lys Ser Phe Asp           630

AAG TGG ACG ACT AAG ATT AAT GAA TGT GTT          1920
Lys Trp Thr Thr Lys Ile Asn Glu Cys Val           640

ATG AAA AAA ATG AAT AAG TTT TAT GAA AAA          1950
Met Lys Lys Met Asn Lys Phe Tyr Glu Lys           650

AGA GGC AAT TTC TAC CAG AAC ACT GTG GGT          1980
Arg Gly Asn Phe Tyr Gln Asn Thr Val Gly           660

GAT CTG CTA AAG TTC ATC CGG AAT TTG GGA          1210
Asp Leu Leu Lys Phe Ile Arg Asn Leu Gly           670

GAA CAC ATT GAT GAA GAA AAG CAT AAA AAG          2040
Glu His Ile Asp Glu Glu Lys His Lys Lys           680
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```
ATG AAA TTA AAA ATT GGA GAC CCT TCC CTG          2070
Met Lys Leu Lys Ile Gly Asp Pro Ser Leu           690

TAT TTT CAG AAG ACA TTT CCA GAT CTG GTG          2100
Tyr Phe Gln Lys Thr Phe Pro Asp Leu Val           700

ATC TAT GTC TAC ACA AAA CTA CAG AAC ACA          2130
Ile Tyr Val Tyr Thr Lys Leu Gln Asn Thr           710

GAA TAT AGA AAG CAT TTC CCC CAA ACC CAC          2160
Glu Tyr Arg Lys His Phe Pro Gln Thr His           720

AGT CCA AAC AAA CCT CAG TGT GAT GGA GCT          2190
Ser Pro Asn Lys Pro Gln Cys Asp Gly Ala           730

GGT GGG GCC AGT GGG TTG GCC AGC CCT GGG          2220
Gly Gly Ala Ser Gly Leu Ala Ser Pro Gly           740

TGC 2223  tgatggactgatttgctggagttcagggaactact    2258
Cys  741

tattagctgtagagtccttggcaaatcacaacat               2292
tctgggcctttaactcaccaggttgcttgtgagggat            2330
gagttgcatagctgatatgtcagtccctggcatcgtg            2367
tattccatatgtctataacaaaagcaatatatacccag           2405
actacactagtccataagctttacccactaactggga            2442
ggacattctgctaagattccttttgtcaattgcaccaa           2480
aagaatgagtgccttgacccctaatgctgcatatgtt            2517
acaattctctcacttaattttcccaatgatcttgcaaa           2555
acagggattatcatccccatttaagaactgaggaacc            2592
tgagactcagagagtgtgagctactggcccaagattat           2630
tcaatttatacctagcactttataaatttatgtggtg            2667
ttattggtacctctcatttgggcaccttaaaacttaac           2705
tatcttccagggctcttccagatgaggcccaaaacat            2742
atatagggttccaggaatctcattcattcattcagta            2780
tttattgagcatctagtataagtctgggcactggatg            2817
catgaatt                                         2825
```

*It is believed that the original codon number 95, i.e. CTT encoding the amino acid number 95, i.e. leucine, is correct, however the alternative codon in parenthesis shown above codon number 95, i.e. CCT encoding the alternative amino acid in parenthesis shown below amino acid number 95, i.e. proline may also exist at this position (see page 81).

SEQ ID NO:1: represents the DNA encoding sequence for the human 2-5A-dependent RNase protein. SEQ ID NO:2: represents the amino acid sequence encoded by the DNA sequence designated SEQ ID NO:1:. SEQ ID NO:3: represents the DNA sequence, represented by SEQ ID NO:1:, having the alternative codon number 95, CCT. SEQ ID NO:4: represents the amino acid sequence encoded by SEQ ID NO:3:, having the alternative amino acid number 95, proline.

TABLE 2

Murine 2-5A-dependent RNase (partial)

SEQ ID NO:5: and SEQ ID NO:6:

-163
attcggcacgaggaaggtgccaattactagctcccttctttattcgtgta

ctgatgagatgtcagaagacagaacataatcagcccaatccctactccaa

gactctcattgtgtcccaaagaaacacacgtgtgcatttcccaaggaaaa

ggcattgaggacc ATG GAG ACC CCG GAT TAT	18
Met Glu Thr Pro Asp Tyr	6
AAC ACA CCT CAG GGT GGA ACC CCA TCA GCG	48
Asn Thr Pro Gln Gly Gly Thr Pro Ser Ala	16
GGA AGT CAG AGG ACC GTT GTC GAA GAT GAT	78
Gly Ser Gln Arg Thr Val Val Glu Asp Asp	26
TCT TCG TTG ATC AAA GCT GTT CAG AAG GGA	108
Ser Ser Leu Ile Lys Ala Val Gln Lys Gly	36
GAT GTT GTC AGG GTC CAG CAA TTG TTA GAA	138
Asp Val Val Arg Val Gln Gln Leu Leu Glu	46
AAA GGG GCT GAT GCC AAT GCC TGT GAA GAC	168
Lys Gly Ala Asp Ala Asn Ala Cys Glu Asp	56
ACC TGG GGC TGG ACA CCT TTG CAC AAC GCA	198
Thr Trp Gly Trp Thr Pro Leu His Asn Ala	66
GTG CAA GCT GGC AGG GTA GAC ATT GTG AAC	228
Val Gln Ala Gly Arg Val Asp Ile Val Asn	76
CTC CTG CTT AGT CAT GGT GCT GAC CCT CAT	258
Leu Leu Leu Ser His Gly Ala Asp Pro His	86
CGG AGG AAG AAG AAT GGG GCC ACC CCC TTC	288
Arg Arg Lys Lys Asn Gly Ala Thr Pro Phe	96
ATC ATT GCT GGG ATC CAG GGA GAT GTG AAA	318
Ile Ile Ala Gly Ile Gln Gly Asp Val Lys	106
CTG CTC GAG ATT CTC CTC TCT TGT GGT GCA	348
Leu Leu Glu Ile Leu Leu Ser Cys Gly Ala	116
GAC GTC AAT GAG TGT GAC GAG AAC GGA TTC	378
Asp Val Asn Glu Cys Asp Glu Asn Gly Phe	126

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ACG GCT TTC ATG GAA GCT GCT GAG CGT GGT          408
Thr Ala Phe Met Glu Ala Ala Glu Arg Gly          136

AAC GCT GAA GCC TTA AGA TTC CTT TTT GCT          438
Asn Ala Glu Ala Leu Arg Phe Leu Phe Ala          146

AAG GGA GCC AAT GTG AAT TTG CGA CGA CAG          468
Lys Gly Ala Asn Val Asn Leu Arg Arg Gln          156

ACA ACG AAG GAC AAA AGG CGA TTG AAG CAA          498
Thr Thr Lys Asp Lys Arg Arg Leu Lys Gln          166

GGA GGC GCC ACA GCT CTC ATG AGC GCT GCT          528
Gly Gly Ala Thr Ala Leu Met Ser Ala Ala          176

GAG AAG GGC CAC CTG GAA GTC CTG AGA ATT          558
Glu Lys Gly His Leu Glu Val Leu Arg Ile          186

CTC CTC AAT GAC ATG AAG GCA GAA GTC GAT          588
Leu Leu Asn Asp Met Lys Ala Glu Val Asp          196

GCT CGG GAC AAC ATG GGC AGA AAT GCC CTG          618
Ala Arg Asp Asn Met Gly Arg Asn Ala Leu          206

ATC CGT ACT CTG CTG AAC TGG GAT TGT GAA          648
Ile Arg Thr Leu Leu Asn Trp Asp Cys Glu          216

AAT GTG GAG GAG ATT ACT TCA ATC CTG ATT          678
Asn Val Glu Glu Ile Thr Ser Ile Leu Ile          226

CAG CAC GGG GCT GAT GTT AAC GTG AGA GGA          708
Gln His Gly Ala Asp Val Asn Val Arg Gly          236

GAA AGA GGG AAA ACA CCC CTC ATC GCA GCA          738
Glu Arg Gly Lys Thr Pro Leu Ile Ala Ala          246

GTG GAG AGG AAG CAC ACA GGC TTG GTG CAG          768
Val Glu Arg Lys His Thr Gly Leu Val Gln          256

ATG CTC CTG AGT CGG GAA GGC ATA AAC ATA          798
Met Leu Leu Ser Arg Glu Gly Ile Asn Ile          266

GAT GCC AGG GAT AAC GAG GGC AAG ACA GCT          828
Asp Ala Arg Asp Asn Glu Gly Lys Thr Ala          276

CTG CTA ATT GCT GTT GAT AAA CAA CTG AAG          858
Leu Leu Ile Ala Val Asp Lys Gln Leu Lys          286

GAA ATT GTC CAG TTG CTT CTT GAA AAG GGA          888
Glu Ile Val Gln Leu Leu Leu Glu Lys Gly          296

GCT GAT AAG TGT GAC GAT CTT GTT TGG ATA          918
Ala Asp Lys Cys Asp Asp Leu Val Trp Ile          306
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GCC AGG AGG AAT CAT GAC TAT CAC CTT GTA                948
Ala Arg Arg Asn His Asp Tyr His Leu Val                316

AAG CTT CTC CTC CCT TAT GTA GCT AAT CCT                978
Lys Leu Leu Leu Pro Tyr Val Ala Asn Pro                326

GAC ACC GAC CCT CCT GCT GGA GAC TGG TCG               1008
Asp Thr Asp Pro Pro Ala Gly Asp Trp Ser                336

CCT CAC AGT TCA CGT TGG GGG ACA GCC TTG               1038
Pro His Ser Ser Arg Trp Gly Thr Ala Leu                346

AAA AGC CTC CAC AGT ATG ACT CGA CCC ATG               1068
Lys Ser Leu His Ser Met Thr Arg Pro Met                356

ATT GGC AAA CTC AAG ATC TTC ATT CAT GAT               1098
Ile Gly Lys Leu Lys Ile Phe Ile His Asp                366

GAC TAT AAA ATT GCT GGC ACT TCC GAA GGG               1128
Asp Tyr Lys Ile Ala Gly Thr Ser Glu Gly                376

GCT GTC TAC CTA GGG ATC TAT GAC AAT CGA               1158
Ala Val Tyr Leu Gly Ile Tyr Asp Asn Arg                386

GAA GTG GCT GTG AAG GTC TTC CGT GAG AAT               1188
Glu Val Ala Val Lys Val Phe Arg Glu Asn                396

AGC CCA CGT GGA TGT AAG GAA GTC TCT TGT               1218
Ser Pro Arg Gly Cys Lys Glu Val Ser Cys                406

CTG CGG GAC TGC GGT GAC CAC AGT AAC TTA               1248
Leu Arg Asp Cys Gly Asp His Ser Asn Leu                416

GTG GCT TTC TAT GGA AGA GAG GAC GAT AAG               1278
Val Ala Phe Tyr Gly Arg Glu Asp Asp Lys                426

GGC TGT TTA TAT GTG TGT GTG TCC CTG TGT               1308
Gly Cys Leu Tyr Val Cys Val Ser Leu Cys                436

GAG TGG ACA CTG GAA GAG TTC CTG AGG TTG               1338
Glu Trp Thr Leu Glu Glu Phe Leu Arg Leu                446

CCC AGA GAG GAA CCT GTG GAG AAC GGG GAA               1368
Pro Arg Glu Glu Pro Val Glu Asn Gly Glu                456

GAT AAG TTT GCC CAC AGC ATC CTA TTA TCT               1398
Asp Lys Phe Ala His Ser Ile Leu Leu Ser                466

ATA TTT GAG GGT GTT CAA AAA CTA CAC TTG               1428
Ile Phe Glu Gly Val Gln Lys Leu His Leu                476

CAT GGA TAT TCC CAT CAG GAC CTG CAA CCA               1458
His Gly Tyr Ser His Gln Asp Leu Gln Pro                486
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CAA AAC ATC TTA ATA GAT TCC AAG AAA GCT          1488
Gln Asn Ile Leu Ile Asp Ser Lys Lys Ala           496

GTC CGG CTG GCA GAT TTT GAT CAG AGC ATC          1518
Val Arg Leu Ala Asp Phe Asp Gln Ser Ile           506

CGA TGG ATG GGA GAG TCA CAG ATG GTC AGG          1548
Arg Trp Met Gly Glu Ser Gln Met Val Arg           516

AGA GAC TTG GAG GAT CTT GGA CGG CTG GTT          1578
Arg Asp Leu Glu Asp Leu Gly Arg Leu Val           526

CTC TAC GTG GTA ATG AAA GGT GAG ATC CCC          1608
Leu Tyr Val Val Met Lys Gly Glu Ile Pro           536

TTT GAG ACA CTA AAG ACT CAG AAT GAT GAA          1638
Phe Glu Thr Leu Lys Thr Gln Asn Asp Glu           546

GTG CTG CTT ACA ATG TCT CCA GAT GAG GAG          1668
Val Leu Leu Thr Met Ser Pro Asp Glu Glu           556

ACT AAG GAC CTC ATT CAT TGC CTG TTT TCT          1698
Thr Lys Asp Leu Ile His Cyc Leu Phe Ser           566

CCT GGA GAA AAT GTC AAG AAC TGC CTG GTA          1728
Pro Gly Glu Asn Val Lys Asn Cys Leu Val           576

GAC CTG CTT GGC CAT CCT TTC TTT TGG ACT          1758
Asp Leu Leu Gly His Pro Phe Phe Trp Thr           586

TGG GAG AAC CGC TAT AGA ACA CTC CGG AAT          1788
Trp Glu Asn Arg Tyr Arg Thr Leu Arg Asn           596

GTG GGA AAT GAA TCT GAC ATC AAA GTA CGG          1818
Val Gly Asn Glu Ser Asp Ile Lys Val Arg           606

AAA TGT AAA AGT GAT CTT CTC AGA CTA CTG          1848
Lys Cys Lys Ser Asp Leu Leu Arg Leu Leu           616

CAG CAT CAG ACA CTT GAG CCT CCC AGA AGC          1878
Gln His Gln Thr Leu Glu Pro Pro Arg Ser           626

TTT GAC CAG TGG ACA TCT AAG ATC GAC AAA          1908
Phe Asp Gln Trp Thr Ser Lys Ile Asp Lys           636

AAT GTT ATG GAT GAA ATG AAT CAT TTC TAC          1938
Asn Val Met Asp Glu Met Asn His Phe Tyr           646

GAA AAG AGA AAA AAA AAC CCT TAT CAG GAT          1968
Glu Lys Arg Lys Lys Asn Pro Tyr Gln Asp           656

ACT GTA GGT GAT CTG CTG AAG TTT ATT CGG          1998
Thr Val Gly Asp Leu Leu Lys Phe Ile Arg           666
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AAT ATA GGC GAA CAC ATC AAT GAG GAA AAA	2028
Asn Ile Gly Glu His Ile Asn Glu Glu Lys	676
AAG CGG GGG	2037
Lys Arg Gly	679

SEQ ID NO:5: represents the DNA sequence encoding Murine 2-5A-dependent RNase (partial). SEQ ID NO:6: represents the amino acid sequence encoded by SEQ ID NO:5:.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Silverman, Robert H.
SenGupta, Dibyendu N.

(ii) TITLE OF INVENTION: Antiviral Transgenic Plants, Vectors, Cells and Methods

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ruden, Barnett, McClosky, Smith, Schuster & Russell
(B) STREET: 200 E. Broward Boulevard
(C) CITY: Fort Lauderdale
(D) STATE: Florida
(E) COUNTRY: USA
(F) ZIP: 33301

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/198,973
(B) FILING DATE: 18-FEB-1994
(C) CLASSIFICATION: 1808

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Manso, Peter J.
(B) REGISTRATION NUMBER: 32,264
(C) REFERENCE/DOCKET NUMBER: CL11363-16

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 305/527/2498
(B) TELEFAX: 305/764/4996

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2928 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 104..2326

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AATCCCAACT TACACTCAAA GCTTCTTTGA TTAAGTGCTA GGAGATAAAT TTGCATTTTC      60

TCAAGGAAAA GGCTAAAAGT GGTAGCAGGT GGCATTTACC GTC ATG GAG AGC AGG        115
                                                Met Glu Ser Arg
                                                 1

GAT CAT AAC AAC CCC CAG GAG GGA CCC ACG TCC TCC AGC GGT AGA AGG        163
Asp His Asn Asn Pro Gln Glu Gly Pro Thr Ser Ser Ser Gly Arg Arg
 5                  10                  15                  20

GCT GCA GTG GAA GAC AAT CAC TTG CTG ATT AAA GCT GTT CAA AAC GAA        211
Ala Ala Val Glu Asp Asn His Leu Leu Ile Lys Ala Val Gln Asn Glu
                25                  30                  35

GAT GTT GAC CTG GTC CAG CAA TTG CTG GAA GGT GGA GCC AAT GTT AAT        259
Asp Val Asp Leu Val Gln Gln Leu Leu Glu Gly Gly Ala Asn Val Asn
            40                  45                  50

TTC CAG GAA GAG GAA GGG GGC TGG ACA CCT CTG CAT AAC GCA GTA CAA        307
Phe Gln Glu Glu Glu Gly Gly Trp Thr Pro Leu His Asn Ala Val Gln
        55                  60                  65

ATG AGC AGG GAG GAC ATT GTG GAA CTT CTG CTT CGT CAT GGT GCT GAC        355
Met Ser Arg Glu Asp Ile Val Glu Leu Leu Leu Arg His Gly Ala Asp
    70                  75                  80

CCT GTT CTG AGG AAG AAG AAT GGG GCC ACG CTT TTT ATC CTC GCA GCG        403
Pro Val Leu Arg Lys Lys Asn Gly Ala Thr Leu Phe Ile Leu Ala Ala
85                  90                  95                  100

ATT GCG GGG AGC GTG AAG CTG CTG AAA CTT TTC CTT TCT AAA GGA GCA        451
Ile Ala Gly Ser Val Lys Leu Leu Lys Leu Phe Leu Ser Lys Gly Ala
                105                 110                 115

GAT GTC AAT GAG TGT GAT TTT TAT GGC TTC ACA GCC TTC ATG GAA GCC        499
Asp Val Asn Glu Cys Asp Phe Tyr Gly Phe Thr Ala Phe Met Glu Ala
            120                 125                 130

GCT GTG TAT GGT AAG GTC AAA GCC CTA AAA TTC CTT TAT AAG AGA GGA        547
Ala Val Tyr Gly Lys Val Lys Ala Leu Lys Phe Leu Tyr Lys Arg Gly
        135                 140                 145

GCA AAT GTG AAT TTG AGG CGA AAG ACA AAG GAG GAT CAA GAG CGG CTG        595
Ala Asn Val Asn Leu Arg Arg Lys Thr Lys Glu Asp Gln Glu Arg Leu
    150                 155                 160

AGG AAA GGA GGG GCC ACA GCT CTC ATG GAC GCT GCT GAA AAA GGA CAC        643
Arg Lys Gly Gly Ala Thr Ala Leu Met Asp Ala Ala Glu Lys Gly His
165                 170                 175                 180

GTA GAG GTC TTG AAG ATT CTC CTT GAT GAG ATG GGG GCA GAT GTA AAC        691
Val Glu Val Leu Lys Ile Leu Leu Asp Glu Met Gly Ala Asp Val Asn
                185                 190                 195

GCC TGT GAC AAT ATG GGC AGA AAT GCC TTG ATC CAT GCT CTC CTG AGC        739
Ala Cys Asp Asn Met Gly Arg Asn Ala Leu Ile His Ala Leu Leu Ser
            200                 205                 210

TCT GAC GAT AGT GAT GTG GAG GCT ATT ACG CAT CTG CTG CTG GAC CAT        787
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Ser Asp Asp Ser Asp Val Glu Ala Ile Thr His Leu Leu Leu Asp His
215                 220                 225

GGG GCT GAT GTC AAT GTG AGG GGA GAA AGA GGG AAG ACT CCC CTG ATC      835
Gly Ala Asp Val Asn Val Arg Gly Glu Arg Gly Lys Thr Pro Leu Ile
230                 235                 240

CTG GCA GTG GAG AAG AAG CAC TTG GGT TTG GTG CAG AGG CTT CTG GAG      883
Leu Ala Val Glu Lys Lys His Leu Gly Leu Val Gln Arg Leu Leu Glu
245                 250                 255                 260

CAA GAG CAC ATA GAG ATT AAT GAC ACA GAC AGT GAT GGC AAA ACA GCA      931
Gln Glu His Ile Glu Ile Asn Asp Thr Asp Ser Asp Gly Lys Thr Ala
                265                 270                 275

CTG CTG CTT GCT GTT GAA CTC AAA CTG AAG AAA ATC GCC GAG TTG CTG      979
Leu Leu Leu Ala Val Glu Leu Lys Leu Lys Lys Ile Ala Glu Leu Leu
            280                 285                 290

TGC AAA CGT GGA GCC AGT ACA GAT TGT GGG GAT CTT GTT ATG ACA GCG     1027
Cys Lys Arg Gly Ala Ser Thr Asp Cys Gly Asp Leu Val Met Thr Ala
295                 300                 305

AGG CGG AAT TAT GAC CAT TCC CTT GTG AAG GTT CTT CTC TCT CAT GGA     1075
Arg Arg Asn Tyr Asp His Ser Leu Val Lys Val Leu Leu Ser His Gly
310                 315                 320

GCC AAA GAA GAT TTT CAC CCT CCT GCT GAA GAC TGG AAG CCT CAG AGC     1123
Ala Lys Glu Asp Phe His Pro Pro Ala Glu Asp Trp Lys Pro Gln Ser
325                 330                 335                 340

TCA CAC TGG GGG GCA GCC CTG AAG GAT CTC CAC AGA ATA TAC CGC CCT     1171
Ser His Trp Gly Ala Ala Leu Lys Asp Leu His Arg Ile Tyr Arg Pro
                345                 350                 355

ATG ATT GGC AAA CTC AAG TTC TTT ATT GAT GAA AAA TAC AAA ATT GCT     1219
Met Ile Gly Lys Leu Lys Phe Phe Ile Asp Glu Lys Tyr Lys Ile Ala
            360                 365                 370

GAT ACT TCA GAA GGA GGC ATC TAC CTG GGG TTC TAT GAG AAG CAA GAA     1267
Asp Thr Ser Glu Gly Gly Ile Tyr Leu Gly Phe Tyr Glu Lys Gln Glu
375                 380                 385

GTA GCT GTG AAG ACG TTC TGT GAG GGC AGC CCA CGT GCA CAG CGG GAA     1315
Val Ala Val Lys Thr Phe Cys Glu Gly Ser Pro Arg Ala Gln Arg Glu
390                 395                 400

GTC TCT TGT CTG CAA AGC AGC CGA GAG AAC AGT CAC TTG GTG ACA TTC     1363
Val Ser Cys Leu Gln Ser Ser Arg Glu Asn Ser His Leu Val Thr Phe
405                 410                 415                 420

TAT GGG AGT GAG AGC CAC AGG GGC CAC TTG TTT GTG TGT GTC ACC CTC     1411
Tyr Gly Ser Glu Ser His Arg Gly His Leu Phe Val Cys Val Thr Leu
                425                 430                 435

TGT GAG CAG ACT CTG GAA GCG TGT TTG GAT GTG CAC AGA GGG GAA GAT     1459
Cys Glu Gln Thr Leu Glu Ala Cys Leu Asp Val His Arg Gly Glu Asp
            440                 445                 450

GTG GAA AAT GAG GAA GAT GAA TTT GCC CGA AAT GTC CTG TCA TCT ATA     1507
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Val Glu Asn Glu Glu Asp Glu Phe Ala Arg Asn Val Leu Ser Ser Ile
      455                 460                 465

TTT AAG GCT GTT CAA GAA CTA CAC TTG TCC TGT GGA TAC ACC CAC CAG      1555
Phe Lys Ala Val Gln Glu Leu His Leu Ser Cys Gly Tyr Thr His Gln
    470                 475                 480

GAT CTG CAA CCA CAA AAC ATC TTA ATA GAT TCT AAG AAA GCT GCT CAC      1603
Asp Leu Gln Pro Gln Asn Ile Leu Ile Asp Ser Lys Lys Ala Ala His
485                 490                 495                 500

CTG GCA GAT TTT GAT AAG AGC ATC AAG TGG GCT GGA GAT CCA CAG GAA      1651
Leu Ala Asp Phe Asp Lys Ser Ile Lys Trp Ala Gly Asp Pro Gln Glu
                505                 510                 515

GTC AAG AGA GAT CTA GAG GAC CTT GGA CGG CTG GTC CTC TAT GTG GTA      1699
Val Lys Arg Asp Leu Glu Asp Leu Gly Arg Leu Val Leu Tyr Val Val
            520                 525                 530

AAG AAG GGA AGC ATC TCA TTT GAG GAT CTG AAA GCT CAA AGT AAT GAA      1747
Lys Lys Gly Ser Ile Ser Phe Glu Asp Leu Lys Ala Gln Ser Asn Glu
        535                 540                 545

GAG GTG GTT CAA CTT TCT CCA GAT GAG GAA ACT AAG GAC CTC ATT CAT      1795
Glu Val Val Gln Leu Ser Pro Asp Glu Glu Thr Lys Asp Leu Ile His
    550                 555                 560

CGT CTC TTC CAT CCT GGG GAA CAT GTG AGG GAC TGT CTG AGT GAC CTG      1843
Arg Leu Phe His Pro Gly Glu His Val Arg Asp Cys Leu Ser Asp Leu
565                 570                 575                 580

CTG GGT CAT CCC TTC TTT TGG ACT TGG GAG AGC CGC TAT AGG ACG CTT      1891
Leu Gly His Pro Phe Phe Trp Thr Trp Glu Ser Arg Tyr Arg Thr Leu
                585                 590                 595

CGG AAT GTG GGA AAT GAA TCC GAC ATC AAA ACA CGA AAA TCT GAA AGT      1939
Arg Asn Val Gly Asn Glu Ser Asp Ile Lys Thr Arg Lys Ser Glu Ser
            600                 605                 610

GAG ATC CTC AGA CTA CTG CAA CCT GGG CCT TCT GAA CAT TCC AAA AGT      1987
Glu Ile Leu Arg Leu Leu Gln Pro Gly Pro Ser Glu His Ser Lys Ser
        615                 620                 625

TTT GAC AAG TGG ACG ACT AAG ATT AAT GAA TGT GTT ATG AAA AAA ATG      2035
Phe Asp Lys Trp Thr Thr Lys Ile Asn Glu Cys Val Met Lys Lys Met
    630                 635                 640

AAT AAG TTT TAT GAA AAA AGA GGC AAT TTC TAC CAG AAC ACT GTG GGT      2083
Asn Lys Phe Tyr Glu Lys Arg Gly Asn Phe Tyr Gln Asn Thr Val Gly
645                 650                 655                 660

GAT CTG CTA AAG TTC ATC CGG AAT TTG GGA GAA CAC ATT GAT GAA GAA      2131
Asp Leu Leu Lys Phe Ile Arg Asn Leu Gly Glu His Ile Asp Glu Glu
                665                 670                 675

AAG CAT AAA AAG ATG AAA TTA AAA ATT GGA GAC CCT TCC CTG TAT TTT      2179
Lys His Lys Lys Met Lys Leu Lys Ile Gly Asp Pro Ser Leu Tyr Phe
            680                 685                 690

CAG AAG ACA TTT CCA GAT CTG GTG ATC TAT GTC TAC ACA AAA CTA CAG      2227
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Gln Lys Thr Phe Pro Asp Leu Val Ile Tyr Val Tyr Thr Lys Leu Gln
       695                 700                 705

AAC ACA GAA TAT AGA AAG CAT TTC CCC CAA ACC CAC AGT CCA AAC AAA      2275
Asn Thr Glu Tyr Arg Lys His Phe Pro Gln Thr His Ser Pro Asn Lys
    710                 715                 720

CCT CAG TGT GAT GGA GCT GGT GGG GCC AGT GGG TTG GCC AGC CCT GGG      2323
Pro Gln Cys Asp Gly Ala Gly Gly Ala Ser Gly Leu Ala Ser Pro Gly
725                 730                 735                 740

TGC TGATGGACTG ATTTGCTGGA GTTCAGGGAA CTACTTATTA GCTGTAGAGT           2376
Cys

CCTTGGCAAA TCACAACATT CTGGGCCTTT TAACTCACCA GGTTGCTTGT GAGGGATGAG    2436

TTGCATAGCT GATATGTCAG TCCCTGGCAT CGTGTATTCC ATATGTCTAT AACAAAAGCA    2496

ATATATACCC AGACTACACT AGTCCATAAG CTTTACCCAC TAACTGGGAG GACATTCTGC    2556

TAAGATTCCT TTTGTCAATT GCACCAAAAG AATGAGTGCC TTGACCCCTA ATGCTGCATA    2616

TGTTACAATT CTCTCACTTA ATTTTCCCAA TGATCTTGCA AAACAGGGAT TATCATCCCC    2676

ATTTAAGAAC TGAGGAACCT GAGACTCAGA GAGTGTGAGC TACTGGCCCA AGATTATTCA    2736

ATTTATACCT AGCACTTTAT AAATTTATGT GGTGTTATTG GTACCTCTCA TTTGGGCACC    2796

TTAAAACTTA ACTATCTTCC AGGGCTCTTC CAGATGAGGC CCAAAACATA TATAGGGGTT    2856

CCAGGAATCT CATTCATTCA TTCAGTATTT ATTGAGCATC TAGTATAAGT CTGGGCACTG    2916

GATGCATGAA TT                                                        2928
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 741 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Glu Ser Arg Asp His Asn Asn Pro Gln Glu Gly Pro Thr Ser Ser
 1               5                  10                  15

Ser Gly Arg Arg Ala Ala Val Glu Asp Asn His Leu Leu Ile Lys Ala
            20                  25                  30

Val Gln Asn Glu Asp Val Asp Leu Val Gln Gln Leu Leu Glu Gly Gly
        35                  40                  45

Ala Asn Val Asn Phe Gln Glu Glu Glu Gly Gly Trp Thr Pro Leu His
    50                  55                  60

Asn Ala Val Gln Met Ser Arg Glu Asp Ile Val Glu Leu Leu Leu Arg
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65 70 75 80

His Gly Ala Asp Pro Val Leu Arg Lys Lys Asn Gly Ala Thr Leu Phe
85 90 95

Ile Leu Ala Ala Ile Ala Gly Ser Val Lys Leu Leu Lys Leu Phe Leu
100 105 110

Ser Lys Gly Ala Asp Val Asn Glu Cys Asp Phe Tyr Gly Phe Thr Ala
115 120 125

Phe Met Glu Ala Ala Val Tyr Gly Lys Val Lys Ala Leu Lys Phe Leu
130 135 140

Tyr Lys Arg Gly Ala Asn Val Asn Leu Arg Arg Lys Thr Lys Glu Asp
145 150 155 160

Gln Glu Arg Leu Arg Lys Gly Gly Ala Thr Ala Leu Met Asp Ala Ala
165 170 175

Glu Lys Gly His Val Glu Val Leu Lys Ile Leu Leu Asp Glu Met Gly
180 185 190

Ala Asp Val Asn Ala Cys Asp Asn Met Gly Arg Asn Ala Leu Ile His
195 200 205

Ala Leu Leu Ser Ser Asp Asp Ser Asp Val Glu Ala Ile Thr His Leu
210 215 220

Leu Leu Asp His Gly Ala Asp Val Asn Val Arg Gly Glu Arg Gly Lys
225 230 235 240

Thr Pro Leu Ile Leu Ala Val Glu Lys Lys His Leu Gly Leu Val Gln
245 250 255

Arg Leu Leu Glu Gln Glu His Ile Glu Ile Asn Asp Thr Asp Ser Asp
260 265 270

Gly Lys Thr Ala Leu Leu Leu Ala Val Glu Leu Lys Leu Lys Lys Ile
275 280 285

Ala Glu Leu Leu Cys Lys Arg Gly Ala Ser Thr Asp Cys Gly Asp Leu
290 295 300

Val Met Thr Ala Arg Arg Asn Tyr Asp His Ser Leu Val Lys Val Leu
305 310 315 320

Leu Ser His Gly Ala Lys Glu Asp Phe His Pro Pro Ala Glu Asp Trp
325 330 335

Lys Pro Gln Ser Ser His Trp Gly Ala Ala Leu Lys Asp Leu His Arg
340 345 350

Ile Tyr Arg Pro Met Ile Gly Lys Leu Lys Phe Phe Ile Asp Glu Lys
355 360 365

Tyr Lys Ile Ala Asp Thr Ser Glu Gly Gly Ile Tyr Leu Gly Phe Tyr
370 375 380

Glu Lys Gln Glu Val Ala Val Lys Thr Phe Cys Glu Gly Ser Pro Arg

385 390 395 400

Ala Gln Arg Glu Val Ser Cys Leu Gln Ser Ser Arg Glu Asn Ser His
405 410 415

Leu Val Thr Phe Tyr Gly Ser Glu Ser His Arg Gly His Leu Phe Val
420 425 430

Cys Val Thr Leu Cys Glu Gln Thr Leu Glu Ala Cys Leu Asp Val His
435 440 445

Arg Gly Glu Asp Val Glu Asn Glu Glu Asp Glu Phe Ala Arg Asn Val
450 455 460

Leu Ser Ser Ile Phe Lys Ala Val Gln Glu Leu His Leu Ser Cys Gly
465 470 475 480

Tyr Thr His Gln Asp Leu Gln Pro Gln Asn Ile Leu Ile Asp Ser Lys
485 490 495

Lys Ala Ala His Leu Ala Asp Phe Asp Lys Ser Ile Lys Trp Ala Gly
500 505 510

Asp Pro Gln Glu Val Lys Arg Asp Leu Glu Asp Leu Gly Arg Leu Val
515 520 525

Leu Tyr Val Val Lys Lys Gly Ser Ile Ser Phe Glu Asp Leu Lys Ala
530 535 540

Gln Ser Asn Glu Glu Val Val Gln Leu Ser Pro Asp Glu Glu Thr Lys
545 550 555 560

Asp Leu Ile His Arg Leu Phe His Pro Gly Glu His Val Arg Asp Cys
565 570 575

Leu Ser Asp Leu Leu Gly His Pro Phe Phe Trp Thr Trp Glu Ser Arg
580 585 590

Tyr Arg Thr Leu Arg Asn Val Gly Asn Glu Ser Asp Ile Lys Thr Arg
595 600 605

Lys Ser Glu Ser Glu Ile Leu Arg Leu Leu Gln Pro Gly Pro Ser Glu
610 615 620

His Ser Lys Ser Phe Asp Lys Trp Thr Thr Lys Ile Asn Glu Cys Val
625 630 635 640

Met Lys Lys Met Asn Lys Phe Tyr Glu Lys Arg Gly Asn Phe Tyr Gln
645 650 655

Asn Thr Val Gly Asp Leu Leu Lys Phe Ile Arg Asn Leu Gly Glu His
660 665 670

Ile Asp Glu Glu Lys His Lys Lys Met Lys Leu Lys Ile Gly Asp Pro
675 680 685

Ser Leu Tyr Phe Gln Lys Thr Phe Pro Asp Leu Val Ile Tyr Val Tyr
690 695 700

Thr Lys Leu Gln Asn Thr Glu Tyr Arg Lys His Phe Pro Gln Thr His

705 710 715 720

Ser Pro Asn Lys Pro Gln Cys Asp Gly Ala Gly Gly Ala Ser Gly Leu
725 730 735

Ala Ser Pro Gly Cys
740

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2928 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 104..2326

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AATCCCAACT TACACTCAAA GCTTCTTTGA TTAAGTGCTA GGAGATAAAT TTGCATTTTC 60

TCAAGGAAAA GGCTAAAAGT GGTAGCAGGT GGCATTTACC GTC ATG GAG AGC AGG 115
Met Glu Ser Arg
1

GAT CAT AAC AAC CCC CAG GAG GGA CCC ACG TCC TCC AGC GGT AGA AGG 163
Asp His Asn Asn Pro Gln Glu Gly Pro Thr Ser Ser Ser Gly Arg Arg
5 10 15 20

GCT GCA GTG GAA GAC AAT CAC TTG CTG ATT AAA GCT GTT CAA AAC GAA 211
Ala Ala Val Glu Asp Asn His Leu Leu Ile Lys Ala Val Gln Asn Glu
25 30 35

GAT GTT GAC CTG GTC CAG CAA TTG CTG GAA GGT GGA GCC AAT GTT AAT 259
Asp Val Asp Leu Val Gln Gln Leu Leu Glu Gly Gly Ala Asn Val Asn
40 45 50

TTC CAG GAA GAG GAA GGG GGC TGG ACA CCT CTG CAT AAC GCA GTA CAA 307
Phe Gln Glu Glu Glu Gly Gly Trp Thr Pro Leu His Asn Ala Val Gln
55 60 65

ATG AGC AGG GAG GAC ATT GTG GAA CTT CTG CTT CGT CAT GGT GCT GAC 355
Met Ser Arg Glu Asp Ile Val Glu Leu Leu Leu Arg His Gly Ala Asp
70 75 80

CCT GTT CTG AGG AAG AAG AAT GGG GCC ACG CCT TTT ATC CTC GCA GCG 403
Pro Val Leu Arg Lys Lys Asn Gly Ala Thr Pro Phe Ile Leu Ala Ala
85 90 95 100

ATT GCG GGG AGC GTG AAG CTG CTG AAA CTT TTC CTT TCT AAA GGA GCA 451
Ile Ala Gly Ser Val Lys Leu Leu Lys Leu Phe Leu Ser Lys Gly Ala
105 110 115

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GAT GTC AAT GAG TGT GAT TTT TAT GGC TTC ACA GCC TTC ATG GAA GCC      499
Asp Val Asn Glu Cys Asp Phe Tyr Gly Phe Thr Ala Phe Met Glu Ala
            120                 125                 130

GCT GTG TAT GGT AAG GTC AAA GCC CTA AAA TTC CTT TAT AAG AGA GGA      547
Ala Val Tyr Gly Lys Val Lys Ala Leu Lys Phe Leu Tyr Lys Arg Gly
        135                 140                 145

GCA AAT GTG AAT TTG AGG CGA AAG ACA AAG GAG GAT CAA GAG CGG CTG      595
Ala Asn Val Asn Leu Arg Arg Lys Thr Lys Glu Asp Gln Glu Arg Leu
    150                 155                 160

AGG AAA GGA GGG GCC ACA GCT CTC ATG GAC GCT GCT GAA AAA GGA CAC      643
Arg Lys Gly Gly Ala Thr Ala Leu Met Asp Ala Ala Glu Lys Gly His
165                 170                 175                 180

GTA GAG GTC TTG AAG ATT CTC CTT GAT GAG ATG GGG GCA GAT GTA AAC      691
Val Glu Val Leu Lys Ile Leu Leu Asp Glu Met Gly Ala Asp Val Asn
                185                 190                 195

GCC TGT GAC AAT ATG GGC AGA AAT GCC TTG ATC CAT GCT CTC CTG AGC      739
Ala Cys Asp Asn Met Gly Arg Asn Ala Leu Ile His Ala Leu Leu Ser
            200                 205                 210

TCT GAC GAT AGT GAT GTG GAG GCT ATT ACG CAT CTG CTG CTG GAC CAT      787
Ser Asp Asp Ser Asp Val Glu Ala Ile Thr His Leu Leu Leu Asp His
        215                 220                 225

GGG GCT GAT GTC AAT GTG AGG GGA GAA AGA GGG AAG ACT CCC CTG ATC      835
Gly Ala Asp Val Asn Val Arg Gly Glu Arg Gly Lys Thr Pro Leu Ile
    230                 235                 240

CTG GCA GTG GAG AAG AAG CAC TTG GGT TTG GTG CAG AGG CTT CTG GAG      883
Leu Ala Val Glu Lys Lys His Leu Gly Leu Val Gln Arg Leu Leu Glu
245                 250                 255                 260

CAA GAG CAC ATA GAG ATT AAT GAC ACA GAC AGT GAT GGC AAA ACA GCA      931
Gln Glu His Ile Glu Ile Asn Asp Thr Asp Ser Asp Gly Lys Thr Ala
                265                 270                 275

CTG CTG CTT GCT GTT GAA CTC AAA CTG AAG AAA ATC GCC GAG TTG CTG      979
Leu Leu Leu Ala Val Glu Leu Lys Leu Lys Lys Ile Ala Glu Leu Leu
            280                 285                 290

TGC AAA CGT GGA GCC AGT ACA GAT TGT GGG GAT CTT GTT ATG ACA GCG     1027
Cys Lys Arg Gly Ala Ser Thr Asp Cys Gly Asp Leu Val Met Thr Ala
        295                 300                 305

AGG CGG AAT TAT GAC CAT TCC CTT GTG AAG GTT CTT CTC TCT CAT GGA     1075
Arg Arg Asn Tyr Asp His Ser Leu Val Lys Val Leu Leu Ser His Gly
    310                 315                 320

GCC AAA GAA GAT TTT CAC CCT CCT GCT GAA GAC TGG AAG CCT CAG AGC     1123
Ala Lys Glu Asp Phe His Pro Pro Ala Glu Asp Trp Lys Pro Gln Ser
325                 330                 335                 340

TCA CAC TGG GGG GCA GCC CTG AAG GAT CTC CAC AGA ATA TAC CGC CCT     1171
Ser His Trp Gly Ala Ala Leu Lys Asp Leu His Arg Ile Tyr Arg Pro
                345                 350                 355
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ATG ATT GGC AAA CTC AAG TTC TTT ATT GAT GAA AAA TAC AAA ATT GCT      1219
Met Ile Gly Lys Leu Lys Phe Phe Ile Asp Glu Lys Tyr Lys Ile Ala
        360                 365                 370

GAT ACT TCA GAA GGA GGC ATC TAC CTG GGG TTC TAT GAG AAG CAA GAA      1267
Asp Thr Ser Glu Gly Gly Ile Tyr Leu Gly Phe Tyr Glu Lys Gln Glu
    375                 380                 385

GTA GCT GTG AAG ACG TTC TGT GAG GGC AGC CCA CGT GCA CAG CGG GAA      1315
Val Ala Val Lys Thr Phe Cys Glu Gly Ser Pro Arg Ala Gln Arg Glu
390                 395                 400

GTC TCT TGT CTG CAA AGC AGC CGA GAG AAC AGT CAC TTG GTG ACA TTC      1363
Val Ser Cys Leu Gln Ser Ser Arg Glu Asn Ser His Leu Val Thr Phe
405                 410                 415                 420

TAT GGG AGT GAG AGC CAC AGG GGC CAC TTG TTT GTG TGT GTC ACC CTC      1411
Tyr Gly Ser Glu Ser His Arg Gly His Leu Phe Val Cys Val Thr Leu
            425                 430                 435

TGT GAG CAG ACT CTG GAA GCG TGT TTG GAT GTG CAC AGA GGG GAA GAT      1459
Cys Glu Gln Thr Leu Glu Ala Cys Leu Asp Val His Arg Gly Glu Asp
        440                 445                 450

GTG GAA AAT GAG GAA GAT GAA TTT GCC CGA AAT GTC CTG TCA TCT ATA      1507
Val Glu Asn Glu Glu Asp Glu Phe Ala Arg Asn Val Leu Ser Ser Ile
    455                 460                 465

TTT AAG GCT GTT CAA GAA CTA CAC TTG TCC TGT GGA TAC ACC CAC CAG      1555
Phe Lys Ala Val Gln Glu Leu His Leu Ser Cys Gly Tyr Thr His Gln
470                 475                 480

GAT CTG CAA CCA CAA AAC ATC TTA ATA GAT TCT AAG AAA GCT GCT CAC      1603
Asp Leu Gln Pro Gln Asn Ile Leu Ile Asp Ser Lys Lys Ala Ala His
485                 490                 495                 500

CTG GCA GAT TTT GAT AAG AGC ATC AAG TGG GCT GGA GAT CCA CAG GAA      1651
Leu Ala Asp Phe Asp Lys Ser Ile Lys Trp Ala Gly Asp Pro Gln Glu
            505                 510                 515

GTC AAG AGA GAT CTA GAG GAC CTT GGA CGG CTG GTC CTC TAT GTG GTA      1699
Val Lys Arg Asp Leu Glu Asp Leu Gly Arg Leu Val Leu Tyr Val Val
        520                 525                 530

AAG AAG GGA AGC ATC TCA TTT GAG GAT CTG AAA GCT CAA AGT AAT GAA      1747
Lys Lys Gly Ser Ile Ser Phe Glu Asp Leu Lys Ala Gln Ser Asn Glu
    535                 540                 545

GAG GTG GTT CAA CTT TCT CCA GAT GAG GAA ACT AAG GAC CTC ATT CAT      1795
Glu Val Val Gln Leu Ser Pro Asp Glu Glu Thr Lys Asp Leu Ile His
550                 555                 560

CGT CTC TTC CAT CCT GGG GAA CAT GTG AGG GAC TGT CTG AGT GAC CTG      1843
Arg Leu Phe His Pro Gly Glu His Val Arg Asp Cys Leu Ser Asp Leu
565                 570                 575                 580

CTG GGT CAT CCC TTC TTT TGG ACT TGG GAG AGC CGC TAT AGG ACG CTT      1891
Leu Gly His Pro Phe Phe Trp Thr Trp Glu Ser Arg Tyr Arg Thr Leu
            585                 590                 595
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CGG AAT GTG GGA AAT GAA TCC GAC ATC AAA ACA CGA AAA TCT GAA AGT      1939
Arg Asn Val Gly Asn Glu Ser Asp Ile Lys Thr Arg Lys Ser Glu Ser
            600                 605                 610

GAG ATC CTC AGA CTA CTG CAA CCT GGG CCT TCT GAA CAT TCC AAA AGT      1987
Glu Ile Leu Arg Leu Leu Gln Pro Gly Pro Ser Glu His Ser Lys Ser
        615                 620                 625

TTT GAC AAG TGG ACG ACT AAG ATT AAT GAA TGT GTT ATG AAA AAA ATG      2035
Phe Asp Lys Trp Thr Thr Lys Ile Asn Glu Cys Val Met Lys Lys Met
    630                 635                 640

AAT AAG TTT TAT GAA AAA AGA GGC AAT TTC TAC CAG AAC ACT GTG GGT      2083
Asn Lys Phe Tyr Glu Lys Arg Gly Asn Phe Tyr Gln Asn Thr Val Gly
645                 650                 655                 660

GAT CTG CTA AAG TTC ATC CGG AAT TTG GGA GAA CAC ATT GAT GAA GAA      2131
Asp Leu Leu Lys Phe Ile Arg Asn Leu Gly Glu His Ile Asp Glu Glu
                665                 670                 675

AAG CAT AAA AAG ATG AAA TTA AAA ATT GGA GAC CCT TCC CTG TAT TTT      2179
Lys His Lys Lys Met Lys Leu Lys Ile Gly Asp Pro Ser Leu Tyr Phe
            680                 685                 690

CAG AAG ACA TTT CCA GAT CTG GTG ATC TAT GTC TAC ACA AAA CTA CAG      2227
Gln Lys Thr Phe Pro Asp Leu Val Ile Tyr Val Tyr Thr Lys Leu Gln
        695                 700                 705

AAC ACA GAA TAT AGA AAG CAT TTC CCC CAA ACC CAC AGT CCA AAC AAA      2275
Asn Thr Glu Tyr Arg Lys His Phe Pro Gln Thr His Ser Pro Asn Lys
    710                 715                 720

CCT CAG TGT GAT GGA GCT GGT GGG GCC AGT GGG TTG GCC AGC CCT GGG      2323
Pro Gln Cys Asp Gly Ala Gly Gly Ala Ser Gly Leu Ala Ser Pro Gly
725                 730                 735                 740

TGC TGATGGACTG ATTTGCTGGA GTTCAGGGAA CTACTTATTA GCTGTAGAGT           2376
Cys

CCTTGGCAAA TCACAACATT CTGGGCCTTT TAACTCACCA GGTTGCTTGT GAGGGATGAG    2436

TTGCATAGCT GATATGTCAG TCCCTGGCAT CGTGTATTCC ATATGTCTAT AACAAAAGCA    2496

ATATATACCC AGACTACACT AGTCCATAAG CTTTACCCAC TAACTGGGAG GACATTCTGC    2556

TAAGATTCCT TTTGTCAATT GCACCAAAAG AATGAGTGCC TTGACCCCTA ATGCTGCATA    2616

TGTTACAATT CTCTCACTTA ATTTTCCCAA TGATCTTGCA AAACAGGGAT TATCATCCCC    2676

ATTTAAGAAC TGAGGAACCT GAGACTCAGA GAGTGTGAGC TACTGGCCCA AGATTATTCA    2736

ATTTATACCT AGCACTTTAT AAATTTATGT GGTGTTATTG GTACCTCTCA TTTGGGCACC    2796

TTAAAACTTA ACTATCTTCC AGGGCTCTTC CAGATGAGGC CCAAAACATA TATAGGGGTT    2856

CCAGGAATCT CATTCATTCA TTCAGTATTT ATTGAGCATC TAGTATAAGT CTGGGCACTG    2916

GATGCATGAA TT                                                        2928
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 741 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Glu Ser Arg Asp His Asn Asn Pro Gln Glu Gly Pro Thr Ser Ser
1 5 10 15

Ser Gly Arg Arg Ala Ala Val Glu Asp Asn His Leu Leu Ile Lys Ala
20 25 30

Val Gln Asn Glu Asp Val Asp Leu Val Gln Gln Leu Leu Glu Gly Gly
35 40 45

Ala Asn Val Asn Phe Gln Glu Glu Glu Gly Gly Trp Thr Pro Leu His
50 55 60

Asn Ala Val Gln Met Ser Arg Glu Asp Ile Val Glu Leu Leu Leu Arg
65 70 75 80

His Gly Ala Asp Pro Val Leu Arg Lys Lys Asn Gly Ala Thr Pro Phe
85 90 95

Ile Leu Ala Ala Ile Ala Gly Ser Val Lys Leu Leu Lys Leu Phe Leu
100 105 110

Ser Lys Gly Ala Asp Val Asn Glu Cys Asp Phe Tyr Gly Phe Thr Ala
115 120 125

Phe Met Glu Ala Ala Val Tyr Gly Lys Val Lys Ala Leu Lys Phe Leu
130 135 140

Tyr Lys Arg Gly Ala Asn Val Asn Leu Arg Arg Lys Thr Lys Glu Asp
145 150 155 160

Gln Glu Arg Leu Arg Lys Gly Gly Ala Thr Ala Leu Met Asp Ala Ala
165 170 175

Glu Lys Gly His Val Glu Val Leu Lys Ile Leu Leu Asp Glu Met Gly
180 185 190

Ala Asp Val Asn Ala Cys Asp Asn Met Gly Arg Asn Ala Leu Ile His
195 200 205

Ala Leu Leu Ser Ser Asp Asp Ser Asp Val Glu Ala Ile Thr His Leu
210 215 220

Leu Leu Asp His Gly Ala Asp Val Asn Val Arg Gly Glu Arg Gly Lys
225 230 235 240

Thr Pro Leu Ile Leu Ala Val Glu Lys Lys His Leu Gly Leu Val Gln
245 250 255

Arg Leu Leu Glu Gln Glu His Ile Glu Ile Asn Asp Thr Asp Ser Asp
260 265 270

Gly Lys Thr Ala Leu Leu Leu Ala Val Glu Leu Lys Leu Lys Lys Ile
275 280 285

Ala Glu Leu Leu Cys Lys Arg Gly Ala Ser Thr Asp Cys Gly Asp Leu
290 295 300

Val Met Thr Ala Arg Arg Asn Tyr Asp His Ser Leu Val Lys Val Leu
305 310 315 320

Leu Ser His Gly Ala Lys Glu Asp Phe His Pro Pro Ala Glu Asp Trp
325 330 335

Lys Pro Gln Ser Ser His Trp Gly Ala Ala Leu Lys Asp Leu His Arg
340 345 350

Ile Tyr Arg Pro Met Ile Gly Lys Leu Lys Phe Phe Ile Asp Glu Lys
355 360 365

Tyr Lys Ile Ala Asp Thr Ser Glu Gly Gly Ile Tyr Leu Gly Phe Tyr
370 375 380

Glu Lys Gln Glu Val Ala Val Lys Thr Phe Cys Glu Gly Ser Pro Arg
385 390 395 400

Ala Gln Arg Glu Val Ser Cys Leu Gln Ser Ser Arg Glu Asn Ser His
405 410 415

Leu Val Thr Phe Tyr Gly Ser Glu Ser His Arg Gly His Leu Phe Val
420 425 430

Cys Val Thr Leu Cys Glu Gln Thr Leu Glu Ala Cys Leu Asp Val His
435 440 445

Arg Gly Glu Asp Val Glu Asn Glu Glu Asp Glu Phe Ala Arg Asn Val
450 455 460

Leu Ser Ser Ile Phe Lys Ala Val Gln Glu Leu His Leu Ser Cys Gly
465 470 475 480

Tyr Thr His Gln Asp Leu Gln Pro Gln Asn Ile Leu Ile Asp Ser Lys
485 490 495

Lys Ala Ala His Leu Ala Asp Phe Asp Lys Ser Ile Lys Trp Ala Gly
500 505 510

Asp Pro Gln Glu Val Lys Arg Asp Leu Glu Asp Leu Gly Arg Leu Val
515 520 525

Leu Tyr Val Val Lys Lys Gly Ser Ile Ser Phe Glu Asp Leu Lys Ala
530 535 540

Gln Ser Asn Glu Glu Val Val Gln Leu Ser Pro Asp Glu Glu Thr Lys
545 550 555 560

Asp Leu Ile His Arg Leu Phe His Pro Gly Glu His Val Arg Asp Cys
565 570 575

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Leu Ser Asp Leu Leu Gly His Pro Phe Phe Trp Thr Trp Glu Ser Arg
            580                 585                 590

Tyr Arg Thr Leu Arg Asn Val Gly Asn Glu Ser Asp Ile Lys Thr Arg
        595                 600                 605

Lys Ser Glu Ser Glu Ile Leu Arg Leu Leu Gln Pro Gly Pro Ser Glu
    610                 615                 620

His Ser Lys Ser Phe Asp Lys Trp Thr Thr Lys Ile Asn Glu Cys Val
625                 630                 635                 640

Met Lys Lys Met Asn Lys Phe Tyr Glu Lys Arg Gly Asn Phe Tyr Gln
                645                 650                 655

Asn Thr Val Gly Asp Leu Leu Lys Phe Ile Arg Asn Leu Gly Glu His
            660                 665                 670

Ile Asp Glu Glu Lys His Lys Lys Met Lys Leu Lys Ile Gly Asp Pro
        675                 680                 685

Ser Leu Tyr Phe Gln Lys Thr Phe Pro Asp Leu Val Ile Tyr Val Tyr
    690                 695                 700

Thr Lys Leu Gln Asn Thr Glu Tyr Arg Lys His Phe Pro Gln Thr His
705                 710                 715                 720

Ser Pro Asn Lys Pro Gln Cys Asp Gly Ala Gly Gly Ala Ser Gly Leu
                725                 730                 735

Ala Ser Pro Gly Cys
            740
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2200 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 164..2200

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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ATTCGGCACG AGGAAGGTGC CAATTACTAG CTCCCTTCTT TATTCGTGTA CTGATGAGAT      60

GTCAGAAGAC AGAACATAAT CAGCCCAATC CCTACTCCAA GACTCTCATT GTGTCCCAAA     120

GAAACACACG TGTGCATTTC CCAAGGAAAA GGCATTGAGG ACC ATG GAG ACC CCG       175
                                                Met Glu Thr Pro
                                                  1

GAT TAT AAC ACA CCT CAG GGT GGA ACC CCA TCA GCG GGA AGT CAG AGG       223
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Asp Tyr Asn Thr Pro Gln Gly Gly Thr Pro Ser Ala Gly Ser Gln Arg
 5                  10                  15                  20

ACC GTT GTC GAA GAT GAT TCT TCG TTG ATC AAA GCT GTT CAG AAG GGA      271
Thr Val Val Glu Asp Asp Ser Ser Leu Ile Lys Ala Val Gln Lys Gly
                25                  30                  35

GAT GTT GTC AGG GTC CAG CAA TTG TTA GAA AAA GGG GCT GAT GCC AAT      319
Asp Val Val Arg Val Gln Gln Leu Leu Glu Lys Gly Ala Asp Ala Asn
            40                  45                  50

GCC TGT GAA GAC ACC TGG GGC TGG ACA CCT TTG CAC AAC GCA GTG CAA      367
Ala Cys Glu Asp Thr Trp Gly Trp Thr Pro Leu His Asn Ala Val Gln
        55                  60                  65

GCT GGC AGG GTA GAC ATT GTG AAC CTC CTG CTT AGT CAT GGT GCT GAC      415
Ala Gly Arg Val Asp Ile Val Asn Leu Leu Leu Ser His Gly Ala Asp
    70                  75                  80

CCT CAT CGG AGG AAG AAG AAT GGG GCC ACC CCC TTC ATC ATT GCT GGG      463
Pro His Arg Arg Lys Lys Asn Gly Ala Thr Pro Phe Ile Ile Ala Gly
85                  90                  95                  100

ATC CAG GGA GAT GTG AAA CTG CTC GAG ATT CTC CTC TCT TGT GGT GCA      511
Ile Gln Gly Asp Val Lys Leu Leu Glu Ile Leu Leu Ser Cys Gly Ala
                105                 110                 115

GAC GTC AAT GAG TGT GAC GAG AAC GGA TTC ACG GCT TTC ATG GAA GCT      559
Asp Val Asn Glu Cys Asp Glu Asn Gly Phe Thr Ala Phe Met Glu Ala
            120                 125                 130

GCT GAG CGT GGT AAC GCT GAA GCC TTA AGA TTC CTT TTT GCT AAG GGA      607
Ala Glu Arg Gly Asn Ala Glu Ala Leu Arg Phe Leu Phe Ala Lys Gly
        135                 140                 145

GCC AAT GTG AAT TTG CGA CGA CAG ACA ACG AAG GAC AAA AGG CGA TTG      655
Ala Asn Val Asn Leu Arg Arg Gln Thr Thr Lys Asp Lys Arg Arg Leu
    150                 155                 160

AAG CAA GGA GGC GCC ACA GCT CTC ATG AGC GCT GCT GAG AAG GGC CAC      703
Lys Gln Gly Gly Ala Thr Ala Leu Met Ser Ala Ala Glu Lys Gly His
165                 170                 175                 180

CTG GAA GTC CTG AGA ATT CTC CTC AAT GAC ATG AAG GCA GAA GTC GAT      751
Leu Glu Val Leu Arg Ile Leu Leu Asn Asp Met Lys Ala Glu Val Asp
                185                 190                 195

GCT CGG GAC AAC ATG GGC AGA AAT GCC CTG ATC CGT ACT CTG CTG AAC      799
Ala Arg Asp Asn Met Gly Arg Asn Ala Leu Ile Arg Thr Leu Leu Asn
            200                 205                 210

TGG GAT TGT GAA AAT GTG GAG GAG ATT ACT TCA ATC CTG ATT CAG CAC      847
Trp Asp Cys Glu Asn Val Glu Glu Ile Thr Ser Ile Leu Ile Gln His
        215                 220                 225

GGG GCT GAT GTT AAC GTG AGA GGA GAA AGA GGG AAA ACA CCC CTC ATC      895
Gly Ala Asp Val Asn Val Arg Gly Glu Arg Gly Lys Thr Pro Leu Ile
    230                 235                 240

GCA GCA GTG GAG AGG AAG CAC ACA GGC TTG GTG CAG ATG CTC CTG AGT      943
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Ala Ala Val Glu Arg Lys His Thr Gly Leu Val Gln Met Leu Leu Ser
245                 250                 255                 260

CGG GAA GGC ATA AAC ATA GAT GCC AGG GAT AAC GAG GGC AAG ACA GCT      991
Arg Glu Gly Ile Asn Ile Asp Ala Arg Asp Asn Glu Gly Lys Thr Ala
                265                 270                 275

CTG CTA ATT GCT GTT GAT AAA CAA CTG AAG GAA ATT GTC CAG TTG CTT     1039
Leu Leu Ile Ala Val Asp Lys Gln Leu Lys Glu Ile Val Gln Leu Leu
            280                 285                 290

CTT GAA AAG GGA GCT GAT AAG TGT GAC GAT CTT GTT TGG ATA GCC AGG     1087
Leu Glu Lys Gly Ala Asp Lys Cys Asp Asp Leu Val Trp Ile Ala Arg
        295                 300                 305

AGG AAT CAT GAC TAT CAC CTT GTA AAG CTT CTC CTC CCT TAT GTA GCT     1135
Arg Asn His Asp Tyr His Leu Val Lys Leu Leu Leu Pro Tyr Val Ala
    310                 315                 320

AAT CCT GAC ACC GAC CCT CCT GCT GGA GAC TGG TCG CCT CAC AGT TCA     1183
Asn Pro Asp Thr Asp Pro Pro Ala Gly Asp Trp Ser Pro His Ser Ser
325                 330                 335                 340

CGT TGG GGG ACA GCC TTG AAA AGC CTC CAC AGT ATG ACT CGA CCC ATG     1231
Arg Trp Gly Thr Ala Leu Lys Ser Leu His Ser Met Thr Arg Pro Met
                345                 350                 355

ATT GGC AAA CTC AAG ATC TTC ATT CAT GAT GAC TAT AAA ATT GCT GGC     1279
Ile Gly Lys Leu Lys Ile Phe Ile His Asp Asp Tyr Lys Ile Ala Gly
            360                 365                 370

ACT TCC GAA GGG GCT GTC TAC CTA GGG ATC TAT GAC AAT CGA GAA GTG     1327
Thr Ser Glu Gly Ala Val Tyr Leu Gly Ile Tyr Asp Asn Arg Glu Val
        375                 380                 385

GCT GTG AAG GTC TTC CGT GAG AAT AGC CCA CGT GGA TGT AAG GAA GTC     1375
Ala Val Lys Val Phe Arg Glu Asn Ser Pro Arg Gly Cys Lys Glu Val
    390                 395                 400

TCT TGT CTG CGG GAC TGC GGT GAC CAC AGT AAC TTA GTG GCT TTC TAT     1423
Ser Cys Leu Arg Asp Cys Gly Asp His Ser Asn Leu Val Ala Phe Tyr
405                 410                 415                 420

GGA AGA GAG GAC GAT AAG GGC TGT TTA TAT GTG TGT GTG TCC CTG TGT     1471
Gly Arg Glu Asp Asp Lys Gly Cys Leu Tyr Val Cys Val Ser Leu Cys
                425                 430                 435

GAG TGG ACA CTG GAA GAG TTC CTG AGG TTG CCC AGA GAG GAA CCT GTG     1519
Glu Trp Thr Leu Glu Glu Phe Leu Arg Leu Pro Arg Glu Glu Pro Val
            440                 445                 450

GAG AAC GGG GAA GAT AAG TTT GCC CAC AGC ATC CTA TTA TCT ATA TTT     1567
Glu Asn Gly Glu Asp Lys Phe Ala His Ser Ile Leu Leu Ser Ile Phe
        455                 460                 465

GAG GGT GTT CAA AAA CTA CAC TTG CAT GGA TAT TCC CAT CAG GAC CTG     1615
Glu Gly Val Gln Lys Leu His Leu His Gly Tyr Ser His Gln Asp Leu
    470                 475                 480

CAA CCA CAA AAC ATC TTA ATA GAT TCC AAG AAA GCT GTC CGG CTG GCA     1663
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Gln Pro Gln Asn Ile Leu Ile Asp Ser Lys Lys Ala Val Arg Leu Ala
485                 490                 495                 500

GAT TTT GAT CAG AGC ATC CGA TGG ATG GGA GAG TCA CAG ATG GTC AGG      1711
Asp Phe Asp Gln Ser Ile Arg Trp Met Gly Glu Ser Gln Met Val Arg
            505                 510                 515

AGA GAC TTG GAG GAT CTT GGA CGG CTG GTT CTC TAC GTG GTA ATG AAA      1759
Arg Asp Leu Glu Asp Leu Gly Arg Leu Val Leu Tyr Val Val Met Lys
        520                 525                 530

GGT GAG ATC CCC TTT GAG ACA CTA AAG ACT CAG AAT GAT GAA GTG CTG      1807
Gly Glu Ile Pro Phe Glu Thr Leu Lys Thr Gln Asn Asp Glu Val Leu
    535                 540                 545

CTT ACA ATG TCT CCA GAT GAG GAG ACT AAG GAC CTC ATT CAT TGC CTG      1855
Leu Thr Met Ser Pro Asp Glu Glu Thr Lys Asp Leu Ile His Cys Leu
550                 555                 560

TTT TCT CCT GGA GAA AAT GTC AAG AAC TGC CTG GTA GAC CTG CTT GGC      1903
Phe Ser Pro Gly Glu Asn Val Lys Asn Cys Leu Val Asp Leu Leu Gly
565                 570                 575                 580

CAT CCT TTC TTT TGG ACT TGG GAG AAC CGC TAT AGA ACA CTC CGG AAT      1951
His Pro Phe Phe Trp Thr Trp Glu Asn Arg Tyr Arg Thr Leu Arg Asn
            585                 590                 595

GTG GGA AAT GAA TCT GAC ATC AAA GTA CGG AAA TGT AAA AGT GAT CTT      1999
Val Gly Asn Glu Ser Asp Ile Lys Val Arg Lys Cys Lys Ser Asp Leu
        600                 605                 610

CTC AGA CTA CTG CAG CAT CAG ACA CTT GAG CCT CCC AGA AGC TTT GAC      2047
Leu Arg Leu Leu Gln His Gln Thr Leu Glu Pro Pro Arg Ser Phe Asp
    615                 620                 625

CAG TGG ACA TCT AAG ATC GAC AAA AAT GTT ATG GAT GAA ATG AAT CAT      2095
Gln Trp Thr Ser Lys Ile Asp Lys Asn Val Met Asp Glu Met Asn His
630                 635                 640

TTC TAC GAA AAG AGA AAA AAA AAC CCT TAT CAG GAT ACT GTA GGT GAT      2143
Phe Tyr Glu Lys Arg Lys Lys Asn Pro Tyr Gln Asp Thr Val Gly Asp
645                 650                 655                 660

CTG CTG AAG TTT ATT CGG AAT ATA GGC GAA CAC ATC AAT GAG GAA AAA      2191
Leu Leu Lys Phe Ile Arg Asn Ile Gly Glu His Ile Asn Glu Glu Lys
            665                 670                 675

AAG CGG GGG                                                         2200
Lys Arg Gly
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 679 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Thr Pro Asp Tyr Asn Thr Pro Gln Gly Gly Thr Pro Ser Ala
1 5 10 15

Gly Ser Gln Arg Thr Val Val Glu Asp Asp Ser Ser Leu Ile Lys Ala
20 25 30

Val Gln Lys Gly Asp Val Val Arg Val Gln Gln Leu Leu Glu Lys Gly
35 40 45

Ala Asp Ala Asn Ala Cys Glu Asp Thr Trp Gly Trp Thr Pro Leu His
50 55 60

Asn Ala Val Gln Ala Gly Arg Val Asp Ile Val Asn Leu Leu Leu Ser
65 70 75 80

His Gly Ala Asp Pro His Arg Arg Lys Lys Asn Gly Ala Thr Pro Phe
85 90 95

Ile Ile Ala Gly Ile Gln Gly Asp Val Lys Leu Leu Glu Ile Leu Leu
100 105 110

Ser Cys Gly Ala Asp Val Asn Glu Cys Asp Glu Asn Gly Phe Thr Ala
115 120 125

Phe Met Glu Ala Ala Glu Arg Gly Asn Ala Glu Ala Leu Arg Phe Leu
130 135 140

Phe Ala Lys Gly Ala Asn Val Asn Leu Arg Arg Gln Thr Thr Lys Asp
145 150 155 160

Lys Arg Arg Leu Lys Gln Gly Gly Ala Thr Ala Leu Met Ser Ala Ala
165 170 175

Glu Lys Gly His Leu Glu Val Leu Arg Ile Leu Leu Asn Asp Met Lys
180 185 190

Ala Glu Val Asp Ala Arg Asp Asn Met Gly Arg Asn Ala Leu Ile Arg
195 200 205

Thr Leu Leu Asn Trp Asp Cys Glu Asn Val Glu Glu Ile Thr Ser Ile
210 215 220

Leu Ile Gln His Gly Ala Asp Val Asn Val Arg Gly Glu Arg Gly Lys
225 230 235 240

Thr Pro Leu Ile Ala Ala Val Glu Arg Lys His Thr Gly Leu Val Gln
245 250 255

Met Leu Leu Ser Arg Glu Gly Ile Asn Ile Asp Ala Arg Asp Asn Glu
260 265 270

Gly Lys Thr Ala Leu Leu Ile Ala Val Asp Lys Gln Leu Lys Glu Ile
275 280 285

Val Gln Leu Leu Leu Glu Lys Gly Ala Asp Lys Cys Asp Asp Leu Val
290 295 300

Trp Ile Ala Arg Arg Asn His Asp Tyr His Leu Val Lys Leu Leu Leu
305 310 315 320

Pro Tyr Val Ala Asn Pro Asp Thr Asp Pro Pro Ala Gly Asp Trp Ser
325 330 335

Pro His Ser Ser Arg Trp Gly Thr Ala Leu Lys Ser Leu His Ser Met
340 345 350

Thr Arg Pro Met Ile Gly Lys Leu Lys Ile Phe Ile His Asp Asp Tyr
355 360 365

Lys Ile Ala Gly Thr Ser Glu Gly Ala Val Tyr Leu Gly Ile Tyr Asp
370 375 380

Asn Arg Glu Val Ala Val Lys Val Phe Arg Glu Asn Ser Pro Arg Gly
385 390 395 400

Cys Lys Glu Val Ser Cys Leu Arg Asp Cys Gly Asp His Ser Asn Leu
405 410 415

Val Ala Phe Tyr Gly Arg Glu Asp Asp Lys Gly Cys Leu Tyr Val Cys
420 425 430

Val Ser Leu Cys Glu Trp Thr Leu Glu Glu Phe Leu Arg Leu Pro Arg
435 440 445

Glu Glu Pro Val Glu Asn Gly Glu Asp Lys Phe Ala His Ser Ile Leu
450 455 460

Leu Ser Ile Phe Glu Gly Val Gln Lys Leu His Leu His Gly Tyr Ser
465 470 475 480

His Gln Asp Leu Gln Pro Gln Asn Ile Leu Ile Asp Ser Lys Lys Ala
485 490 495

Val Arg Leu Ala Asp Phe Asp Gln Ser Ile Arg Trp Met Gly Glu Ser
500 505 510

Gln Met Val Arg Arg Asp Leu Glu Asp Leu Gly Arg Leu Val Leu Tyr
515 520 525

Val Val Met Lys Gly Glu Ile Pro Phe Glu Thr Leu Lys Thr Gln Asn
530 535 540

Asp Glu Val Leu Leu Thr Met Ser Pro Asp Glu Glu Thr Lys Asp Leu
545 550 555 560

Ile His Cys Leu Phe Ser Pro Gly Glu Asn Val Lys Asn Cys Leu Val
565 570 575

Asp Leu Leu Gly His Pro Phe Phe Trp Thr Trp Glu Asn Arg Tyr Arg
580 585 590

Thr Leu Arg Asn Val Gly Asn Glu Ser Asp Ile Lys Val Arg Lys Cys
595 600 605

Lys Ser Asp Leu Leu Arg Leu Leu Gln His Gln Thr Leu Glu Pro Pro
610 615 620

Arg Ser Phe Asp Gln Trp Thr Ser Lys Ile Asp Lys Asn Val Met Asp
625 630 635 640

Glu Met Asn Asn Phe Tyr Glu Lys Arg Lys Lys Asn Pro Tyr Gln Asp
645 650 655

Thr Val Gly Asp Leu Leu Lys Phe Ile Arg Asn Ile Gly Glu His Ile
660 665 670

Asn Glu Glu Lys Lys Arg Gly
675

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 190 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Arg Arg Lys Pro Arg Gln Asn Asn Arg Arg Asp Arg Asn Glu Arg
1 5 10 15

Arg Asp Thr Arg Ser Glu Arg Thr Glu Gly Ser Asp Asn Arg Glu Glu
20 25 30

Asn Arg Arg Asn Arg Arg Gln Ala Gln Gln Gln Thr Ala Glu Thr Arg
35 40 45

Glu Ser Arg Gln Gln Ala Glu Val Thr Glu Lys Ala Arg Thr Ala Asp
50 55 60

Glu Gln Gln Ala Pro Arg Arg Glu Arg Ser Arg Arg Arg Asn Asp Asp
65 70 75 80

Lys Arg Gln Ala Gln Gln Glu Ala Lys Ala Leu Asn Val Glu Glu Gln
85 90 95

Ser Val Gln Glu Thr Glu Gln Glu Glu Arg Val Arg Pro Val Gln Pro
100 105 110

Arg Arg Lys Gln Arg Gln Leu Asn Gln Lys Val Arg Tyr Glu Gln Ser
115 120 125

Val Ala Glu Glu Ala Val Val Ala Pro Val Val Glu Glu Thr Val Ala
130 135 140

Ala Glu Pro Ile Val Gln Glu Ala Pro Ala Pro Arg Thr Glu Leu Val
145 150 155 160

Lys Val Pro Leu Pro Val Val Ala Gln Thr Ala Pro Glu Gln Gln Glu
165 170 175

Glu Asn Asn Ala Asp Asn Arg Asp Asn Gly Gly Met Pro Ser

180 185 190

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2562 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CAGTTTCTGG AGCAAATTCA GTTTGCCTTC CTGGATTTGT AAATTGTAAT GACCTCAAAA 60

CTTTAGCAGT TCTTCCATCT GACTCAGGTT TGCTTCTCTG GCGGTCTTCA GAATCAACAT 120

CCACACTTCC GTGATTATCT GCGTGCATTT TGGACAAAGC TTCCAACCAG GATACGGGAA 180

GAAGAAATGG CTGGTGATCT TTCAGCAGGT TTCTTCATGG AGGAACTTAA TACATACCGT 240

CAGAAGCAGG GAGTAGTACT TAAATATCAA GAACTGCCTA ATTCAGGACC TCCACATGAT 300

AGGAGGTTTA CATTTCAAGT TATAATAGAT GGAAGAGAAT TTCCAGAAGG TGAAGGTAGA 360

TCAAAGAAGG AAGCAAAAAA TGCCGCAGCC AAATTAGCTG TTGAGATACT TAATAAGGAA 420

AAGAAGGCAG TTAGTCCTTT ATTATTGACA ACAACGAATT CTTCAGAAGG ATTATCCATG 480

GGGAATTACA TAGGCCTTAT CAATAGAATT GCCCAGAAGA AAAGACTAAC TGTAAATTAT 540

GAACAGTGTG CATCGGGGGT GCATGGGCCA GAAGGATTTC ATTATAAATG CAAAATGGGA 600

CAGAAAGAAT ATAGTATTGG TACAGGTTCT ACTAAACAGG AAGCAAAACA ATTGGCCGCT 660

AAACTTGCAT ATCTTCAGAT ATTATCAGAA GAAACCTCAG TGAAATCTGA CTACCTGTCC 720

TCTGGTTCTT TTGCTACTAC GTGTGAGTCC CAAAGCAACT CTTTAGTGAC CAGCACACTC 780

GCTTCTGAAT CATCATCTGA AGGTGACTTC TCAGCAGATA CATCAGAGAT AAATTCTAAC 840

AGTGACAGTT TAAACAGTTC TTCGTTGCTT ATGAATGGTC TCAGAAATAA TCAAAGGAAG 900

GCAAAAAGAT CTTTGGCACC CAGATTTGAC CTTCCTGACA TGAAAGAAAC AAAGTATACT 960

GTGGACAAGA GGTTTGGCAT GGATTTTAAA GAAATAGAAT TAATTGGCTC AGGTGGATTT 1020

GGCCAAGTTT TCAAAGCAAA ACACAGAATT GACGGAAAGA CTTACGTTAT TAAACGTGTT 1080

AAATATAATA ACGAGAAGGC GGAGCGTGAA GTAAAAGCAT TGGCAAAACT TGATCATGTA 1140

AATATTGTTC ACTACAATGG CTGTTGGGAT GGATTTGATT ATGATCCTGA GACCAGTGAT 1200

GATTCTCTTG AGAGCAGTGA TTATGATCCT GAGAACAGCA AAAATAGTTC AAGGTCAAAG 1260

ACTAAGTGCC TTTTCATCCA AATGGAATTC TGTGATAAAG GGACCTTGGA ACAATGGATT 1320

GAAAAAAGAA GAGGCGAGAA ACTAGACAAA GTTTTGGCTT TGGAACTCTT TGAACAAATA 1380

ACAAAAGGGG TGGATTATAT ACATTCAAAA AAATTAATTC ATAGAGATCT TAAGCCAAGT 1440

AATATATTCT TAGTAGATAC AAAACAAGTA AAGATTGGAG ACTTTGGACT TGTAACATCT 1500

CTGAAAAATG ATGGAAAGCG AACAAGGAGT AGGGGAACTT TGCGATACAT GAGCCCAGAA 1560

CAGATTTCTT CGCAAGACTA TGGAAAGGAA GTGGACCTCT ACGCTTTGGG GCTAATTCTT 1620

GCTGAACTTC TTCATGTATG TGACACTGCT TTTGAAACAT CAAAGTTTTT CACAGACCTA 1680

CGGGATGGCA TCATCTCAGA TATATTTGAT AAAAAAGAAA AAACTCTTCT ACAGAAATTA 1740

CTCTCAAAGA AACCTGAGGA TCGACCTAAC ACATCTGAAA TACTAAGGAC CTTGACTGTG 1800

TGGAAGAAAA GCCCAGAGAA AAATGAACGA CACACATGTT AGAGCCCTTC TGAAAAAGTA 1860

TCCTGCTTCT GATATGCAGT TTTCCTTAAA TTATCTAAAA TCTGCTAGGG AATATCAATA 1920

GATATTTACC TTTTATTTTA ATGTTTCCTT TAATTTTTTA CTATTTTTAC TAATCTTTCT 1980

GCAGAAACAG AAAGGTTTTC TTCTTTTTGC TTCAAAAACA TTCTTACATT TTACTTTTTC 2040

CTGGCTCATC TCTTTATTTT TTTTTTTTTT TTTTAAAGAC AGAGTCTCGC TCTGTTGCCC 2100

AGGCTGGAGT GCAATGACAC AGTCTTGGCT CACTGCAACT TCTGCCTCTT GGGTTCAAGT 2160

GATTCTCCTG CCTCAGCCTC CTGAGTAGCT GGATTACAGG CATGTGCCAC CCACCCAACT 2220

AATTTTTGTG TTTTTAATAA AGACAGGGTT TCACCATGTT GGCCAGGCTG GTCTCAAACT 2280

CCTGACCTCA AGTAATCCAC CTGCCTCGGC CTCCCAAAGT GCTGGGATTA CAGGGATGAG 2340

CCACCGCGCC CAGCCTCATC TCTTTGTTCT AAAGATGGAA AAACCACCCC CAAATTTTCT 2400

TTTTATACTA TTAATGAATC AATCAATTCA TATCTATTTA TTAAATTTCT ACCGCTTTTA 2460

GGCCAAAAAA ATGTAAGATC GTTCTCTGCC TCACATAGCT TACAAGCCAG CTGGAGAAAT 2520

ATGGTACTCA TTAAAAAAAA AAAAAAAAAG TGATGTACAA CC 2562

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 551 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ala Gly Asp Leu Ser Ala Gly Phe Phe Met Glu Glu Leu Asn Thr
1 5 10 15

Tyr Arg Gln Lys Gln Gly Val Val Leu Lys Tyr Gln Glu Leu Pro Asn

20 25 30

Ser Gly Pro Pro His Asp Arg Arg Phe Thr Phe Gln Val Ile Ile Asp
35 40 45

Gly Arg Glu Phe Pro Glu Gly Glu Gly Arg Ser Lys Lys Glu Ala Lys
50 55 60

Asn Ala Ala Ala Lys Leu Ala Val Glu Ile Leu Asn Lys Glu Lys Lys
65 70 75 80

Ala Val Ser Pro Leu Leu Leu Thr Thr Thr Asn Ser Ser Glu Gly Leu
85 90 95

Ser Met Gly Asn Tyr Ile Gly Leu Ile Asn Arg Ile Ala Gln Lys Lys
100 105 110

Arg Leu Thr Val Asn Tyr Glu Gln Cys Ala Ser Gly Val His Gly Pro
115 120 125

Glu Gly Phe His Tyr Lys Cys Lys Met Gly Gln Lys Glu Tyr Ser Ile
130 135 140

Gly Thr Gly Ser Thr Lys Gln Glu Ala Lys Gln Leu Ala Ala Lys Leu
145 150 155 160

Ala Tyr Leu Gln Ile Leu Ser Glu Glu Thr Ser Val Lys Ser Asp Tyr
165 170 175

Leu Ser Ser Gly Ser Phe Ala Thr Thr Cys Glu Ser Gln Ser Asn Ser
180 185 190

Leu Val Thr Ser Thr Leu Ala Ser Glu Ser Ser Ser Glu Gly Asp Phe
195 200 205

Ser Ala Asp Thr Ser Glu Ile Asn Ser Asn Ser Asp Ser Leu Asn Ser
210 215 220

Ser Ser Leu Leu Met Asn Gly Leu Arg Asn Asn Gln Arg Lys Ala Lys
225 230 235 240

Arg Ser Leu Ala Pro Arg Phe Asp Leu Pro Asp Met Lys Glu Thr Lys
245 250 255

Tyr Thr Val Asp Lys Arg Phe Gly Met Asp Phe Lys Glu Ile Glu Leu
260 265 270

Ile Gly Ser Gly Gly Phe Gly Gln Val Phe Lys Ala Lys His Arg Ile
275 280 285

Asp Gly Lys Thr Tyr Val Ile Lys Arg Val Lys Tyr Asn Asn Glu Lys
290 295 300

Ala Glu Arg Glu Val Lys Ala Leu Ala Lys Leu Asp His Val Asn Ile
305 310 315 320

Val His Tyr Asn Gly Cys Trp Asp Gly Phe Asp Tyr Asp Pro Glu Thr
325 330 335

Ser Asp Asp Ser Leu Glu Ser Ser Asp Tyr Asp Pro Glu Asn Ser Lys

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                340                 345                 350
Asn Ser Ser Arg Ser Lys Thr Lys Cys Leu Phe Ile Gln Met Glu Phe
        355                 360                 365
Cys Asp Lys Gly Thr Leu Glu Gln Trp Ile Glu Lys Arg Arg Gly Glu
    370                 375                 380
Lys Leu Asp Lys Val Leu Ala Leu Glu Leu Phe Glu Gln Ile Thr Lys
385                 390                 395                 400
Gly Val Asp Tyr Ile His Ser Lys Lys Leu Ile His Arg Asp Leu Lys
                405                 410                 415
Pro Ser Asn Ile Phe Leu Val Asp Thr Lys Gln Val Lys Ile Gly Asp
            420                 425                 430
Phe Gly Leu Val Thr Ser Leu Lys Asn Asp Gly Lys Arg Thr Arg Ser
        435                 440                 445
Lys Gly Thr Leu Arg Tyr Met Ser Pro Glu Gln Ile Ser Ser Gln Asp
    450                 455                 460
Tyr Gly Lys Glu Val Asp Leu Tyr Ala Leu Gly Leu Ile Leu Ala Glu
465                 470                 475                 480
Leu Leu His Val Cys Asp Thr Ala Phe Glu Thr Ser Lys Phe Phe Thr
                485                 490                 495
Asp Leu Arg Asp Gly Ile Ile Ser Asp Ile Phe Asp Lys Lys Glu Lys
            500                 505                 510
Thr Leu Leu Gln Lys Leu Leu Ser Lys Lys Pro Glu Asp Arg Pro Asn
        515                 520                 525
Thr Ser Glu Ile Leu Arg Thr Leu Thr Val Trp Lys Lys Ser Pro Glu
    530                 535                 540
Lys Asn Glu Arg His Thr Cys
545                 550
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1650 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
AACTGAAACC AACAGCAGTC CAAGCTCAGT CAGCAGAAGA GATAAAAGCA AACAGGTCTG      60
GGAGGCAGTT CTGTTGCCAC TCTCTCTCCT GTCAATGATG GATCTCAGAA ATACCCCAGC     120
CAAATCTCTG GACAAGTTCA TTGAAGACTA TCTCTTGCCA GACACGTGTT TCCGCATGCA     180
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AATCGACCAT GCCATTGACA TCATCTGTGG GTTCCTGAAG GAAAGGTGCT TCCGAGGTAG 240

CTCCTACCCT GTGTGTGTGT CCAAGGTGGT AAAGGGTGGC TCCTCAGGCA AGGGCACCAC 300

CCTCAGAGGC CGATCTGACG CTGACCTGGT TGTCTTCCTC AGTCCTCTCA GCACTTTTCA 360

GGATCAGTTA AATCGCCGGG GAGAGTTCAT CCAGGAAATT AGGAGACAGC TGGAAGCCTG 420

TCAAAGAGAG AGAGCACTTT CCGTGAAGTT TGAGGTCCAG GCTCCACGCT GGGGCAACCC 480

CCGTGCGCTC AGCTTCGTAC TGAGTTCGCT CCAGCTCGGG GAGGGGGTGG AGTTCGATGT 540

GCTGCCTGCC TTTGATGCCC TGGGTCAGTT GACTGGCAGC TATAAACCTA ACCCCCAAAT 600

CTATGTCAAG CTCATCGAGG AGTGCACCGA CCTGCAGAAA GAGGGCGAGT TCTCCACCTG 660

CTTCACAGAA CTACAGAGAG ACTTCCTGAA GCAGCGCCCC ACCAAGCTCA AGAGCCTCAT 720

CCGCCTAGTC AAGCACTGGT ACCAAAATTG TAAGAAGAAG CTTGGGAAGC TGCCACCTCA 780

GTATGCCCTG GAGCTCCTGA CGGTCTATGC TTGGGAGCGA GGGAGCATGA AAACACATTT 840

CAACACAGCC CAAGGATTTC GGACGGTCTT GGAATTAGTC ATAAACTACC AGCAACTCTG 900

CATCTACTGG ACAAAGTATT ATGACTTTAA AAACCCCATT ATTGAAAAGT ACCTGAGAAG 960

GCAGCTCACG AAACCCAGGC CTGTGATCCT GGACCCGGCG GACCCTACAG GAAACTTGGG 1020

TGGTGGAGAC CCAAAGGGTT GGAGGCAGCT GGCACAAGAG GCTGAGGCCT GGCTGAATTA 1080

CCCATGCTTT AAGAATTGGG ATGGGTCCCC AGTGAGCTCC TGGATTCTGC TGGCTGAAAG 1140

CAACAGTACA GACGATGAGA CCGACGATCC CAGGACGTAT CAGAAATATG GTTACATTGG 1200

AACACATGAG TACCCTCATT TCTCTCATAG ACCCAGCACG CTCCAGGCAG CATCCACCCC 1260

ACAGGCAGAA GAGGACTGGA CCTGCACCAT CCTCTGAATG CCAGTGCATC TTGGGGGAAA 1320

GGGCTCCAGT GTTATCTGGA CCAGTTCCTT CATTTTCAGG TGGGACTCTT GATCCAGAGA 1380

AGACAAAGCT CCTCAGTGAG CTGGTGTATA ATCCAAGACA GAACCCAAGT CTCCTGACTC 1440

CTGGCCTTCT ATGCCCTCTA TCCTATCATA GATAACATTC TCCACAGCCT CACTTCATTC 1500

CACCTATTCT CTGAAAATAT TCCCTGAGAG AGAACAGAGA GATTTAGATA AGAGAATGAA 1560

ATTCCAGCCT TGACTTTCTT CTGTGCACCT GATGGGAGGG TAATGTCTAA TGTATTATCA 1620

ATAACAATAA AAATAAAGCA AATACCAAAA 1650

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Met Asp Leu Arg Asn Thr Pro Ala Lys Ser Leu Asp Lys Phe Ile
1 5 10 15

Glu Asp Tyr Leu Leu Pro Asp Thr Cys Phe Arg Met Gln Ile Asp His
20 25 30

Ala Ile Asp Ile Ile Cys Gly Phe Leu Lys Glu Arg Cys Phe Arg Gly
35 40 45

Ser Ser Tyr Pro Val Cys Val Ser Lys Val Val Lys Gly Gly Ser Ser
50 55 60

Gly Lys Gly Thr Thr Leu Arg Gly Arg Ser Asp Ala Asp Leu Val Val
65 70 75 80

Phe Leu Ser Pro Leu Thr Thr Phe Gln Asp Gln Leu Asn Arg Arg Gly
85 90 95

Glu Phe Thr Gln Glu Ile Arg Arg Gln Leu Glu Ala Cys Gln Arg Glu
100 105 110

Arg Ala Leu Ser Val Lys Phe Glu Val Gln Ala Pro Arg Trp Gly Asn
115 120 125

Pro Arg Ala Leu Ser Phe Val Leu Ser Ser Leu Gln Leu Gly Glu Gly
130 135 140

Val Glu Phe Asp Val Leu Pro Ala Phe Asp Ala Leu Gly Gln Leu Thr
145 150 155 160

Gly Ser Tyr Lys Pro Asn Pro Gln Ile Tyr Val Lys Leu Ile Glu Glu
165 170 175

Cys Thr Asp Leu Gln Lys Glu Gly Glu Phe Ser Thr Cys Gly Thr Glu
180 185 190

Leu Gln Arg Asp Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser Leu
195 200 205

Ile Arg Leu Val Lys His Trp Thr Gln Asn Cys Lys Lys Lys Leu Gly
210 215 220

Lys Leu Pro Pro Gln Tyr Ala Leu Glu Leu Leu Thr Val Tyr Ala Trp
225 230 235 240

Glu Arg Gly Ser Met Lys Thr His Phe Asn Thr Ala Gln Gly Phe Arg
245 250 255

Thr Val Leu Glu Leu Val Ile Asn Tyr Gln Gln Leu Cys Ile Tyr Trp
260 265 270

Ile Lys Tyr Tyr Asp Phe Lys Asn Pro Ile Ile Glu Lys Tyr Leu Arg
275 280 285

Arg Gln Leu Thr Lys Pro Arg Pro Val Ile Leu Lys Pro Ala Asp Pro

290 295 300

Thr Gly Asn Leu Gly Gly Gly Asp Pro Lys Gly Trp Arg Gln Leu Ala
305 310 315 320

Gln Glu Ala Glu Ala Trp Leu Asn Tyr Pro Cys Phe Lys Asn Trp Asp
325 330 335

Gly Ser Pro Val Ser Ser Trp Ile Leu Leu Ala Glu Ser Asn Ser Thr
340 345 350

Asp Asp Glu Thr Asp Asp Pro Arg Thr Tyr Gln Lys Tyr Gly Tyr Ile
355 360 365

Gly Thr His Glu Tyr Pro His Phe Ser His Arg Pro Ser Thr Leu Gln
370 375 380

Ala Ala Ser Thr Pro Gln Ala Glu Glu Asp Trp Thr Cys Thr Ile Leu
385 390 395 400

The present invention may, of course, be carried out in other specific ways than those herein set forth without departing from the spirit and essential characteristics of the invention. For example, the nucleotide sequences disclosed herein may be combined with other nucleotide sequences to generate heterologous nucleotide sequences for introduction into the genomes of plants to form transgenic plants. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive and all changes coming within the meaning and equivalency range of the appended claims are intended to be embraced herein.

Having described our invention, we claim:

1. A transgenic plant all of whose cells contain at least one nucleotide sequence introduced into said transgenic plant, or ancestor of said transgenic plant, said introduced nucleotide sequence encoding an amino acid sequence having antiviral activity for conferring to the transgenic plant immunity or resistance against viral infection.

2. A transgenic plant of claim 1, said nucleotide sequence includes the nucleotides designated as 1-2223 in Table 1 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase.

3. A transgenic plant of claim 1, said nucleotide sequence being selected from a group consisting of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, 1-1028 and 1-884 in Table 2.

4. A transgenic plant of claim 1, said nucleotide sequence includes the nucleotides designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

5. A transgenic plant of claim 1, said nucleotide sequence includes the nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase.

6. A transgenic plant of claim 1, said amino acid sequence having activity similar or identical to 2-5A-dependent RNase.

7. A transgenic plant of claim 1, said amino acid sequence having activity similar or identical to 2-5A synthetase.

8. A transgenic plant of claim 1, said amino acid sequence having activity similar or identical to PKR.

9. A transgenic plant of claim 1, said amino acid sequence having activity similar or identical to 2-5A-dependent RNase, said nucleotide sequence further encoding a second amino acid sequence, said second amino acid sequence having activity similar or identical to 2-5A synthetase.

10. A transgenic plant of claim 9, said nucleotide sequence includes nucleotides designated as 1-2223 in Table 1 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase and nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase.

11. A transgenic plant of claim 9, said nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase and nucleotides selected from the group consisting of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, 1-1028 and 1-884 in Table 2.

12. A transgenic plant of claim 9, said nucleotide sequence further encoding a third amino acid sequence, said third amino acid sequence having activity similar or identical to PKR.

13. A transgenic tobacco plant of claim 12, said nucleotide sequence including nucleotides designated as 1-2223 in Table 1 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase, nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase and nucleotides designated as 1-2562 in FIG. 18 or any part of said nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

14. A transgenic plant of claim 11, said nucleotide sequence including nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase, nucleotides designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR and nucleotides selected from the group of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, 1-1028, and 1-884 in Table 2.

15. A transgenic plant of claim 1, said amino acid sequence having activity similar or identical to 2-5A synthetase, said nucleotide sequence further encoding a second amino acid sequence, said amino acid sequence having activity similar or identical to PKR.

16. A transgenic plant of claim 15, said nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase and nucleotides designated as 1-2562 in FIG. 18 or any part of said nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

17. A transgenic plant of claim 1, said amino acid sequence having activity similar or identical to 2-5A-dependent RNase, said nucleotide sequence further encoding a second amino acid sequence, said amino acid sequence having activity similar or identical to PKR.

18. A transgenic plant of claim 17, said nucleotide sequence includes nucleotides designated as 1-2223 in Table 1 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase and designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

19. A transgenic plant of claim 17, said nucleotide sequence includes nucleotides designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR and nucleotides selected from a group consisting of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, 1-1028 and 1-884 in Table 2.

20. A transgenic plant of claim 1, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

21. A transgenic plant of claim 2, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

22. A transgenic plant of claim 3, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

23. A transgenic plant of claim 4, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

24. A transgenic plant of claim 5, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

25. A transgenic plant of claim 6, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

26. A transgenic plant of claim 7, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

27. A transgenic plant of claim 8, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

28. A transgenic plant of claim 9, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

29. A transgenic plant of claim 12, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

30. A transgenic plant of claim 15, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

31. A transgenic plant of claim 17, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

32. A transgenic tobacco plant all of whose cells contain a nucleotide sequence introduced into said transgenic tobacco plant, or an ancestor of said transgenic tobacco plant, said nucleotide sequence encoding an amino acid sequence having activity similar or identical to 2-5A-dependent RNase.

33. A transgenic tobacco plant of claim 32, said nucleotide sequence includes nucleotides designated as 1-2223 in Table 1 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase.

34. A transgenic tobacco plant of claim 32, said nucleotide sequence includes nucleotides selected from the group of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, 1-1028 and 1-884 in Table 2.

35. A transgenic tobacco plant all of whose cells contain a nucleotide sequence introduced into said transgenic tobacco plant, or an ancestor of said transgenic tobacco plant, said nucleotide sequence encoding an amino acid sequence having activity similar or identical to 2-5A-synthetase.

36. A transgenic tobacco plant of claim 35, said nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase.

37. A transgenic tobacco plant all of whose cells contain a nucleotide sequence introduced into said transgenic tobacco plant, or an ancestor of said transgenic tobacco plant, said nucleotide sequence encoding an amino acid sequence having activity similar or identical to PKR.

38. A transgenic tobacco plant of claim 37, said nucleotide sequence includes nucleotides designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

39. A transgenic plant of claim 1, said transgenic plant all of whose cells contain at least three nucleotide sequences, each said nucleotide sequence being introduced into said transgenic plant, or an ancestor of said transgenic plant, said first introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to 2-5A-dependent RNase, said second introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to 2-5A synthetase, and said third introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to PKR.

40. A transgenic plant of claim 39, said transgenic plant being a transgenic tobacco plant.

41. A transgenic plant of claim 39, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

42. A transgenic plant of claim 39, said first nucleotide sequence including nucleotides designated as 1-2223 in Table I or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase.

43. A transgenic plant of claim 42, said second nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase and said third nucleotide sequence includes nucleotides designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

44. A transgenic plant of claim 39, said first nucleotide sequence includes nucleotides selected from a group consisting of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, 1-1028 and 1-884 in Table 2.

45. A transgenic plant of claim 44, said second nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase, and said third nucleotide sequence includes nucleotides designated as 1-2562 in FIG. 18 or any part of said nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

46. A transgenic plant of claim 42, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

47. A transgenic plant of claim 43, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

48. A transgenic plant of claim 44, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

49. A transgenic plant of claim 45, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

50. A transgenic plant of claim 1, said transgenic plant all of whose cells contain at least two nucleotide sequences, each said nucleotide sequence being introduced into said transgenic plant, or an ancestor of said transgenic plant, said first introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to 2-5A-dependent RNase, and said second introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to 2-5A synthetase.

51. A transgenic plant of claim 50, said transgenic plant being a transgenic tobacco plant.

52. A transgenic plant of claim 50, said first nucleotide sequence includes nucleotides designated as 1-2223 in Table 1 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase.

53. A transgenic plant of claim 52, said second nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase.

54. A transgenic plant of claim 50, said first nucleotide sequence includes nucleotides selected from a group consisting of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, 1-1028 and 1-884 in Table 2.

55. A transgenic plant of claim 54, said second nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase.

56. A transgenic plant of claim 50, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

57. A transgenic plant of claim 52, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

58. A transgenic plant of claim 53, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

59. A transgenic plant of claim 54, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

60. A transgenic plant of claim 55, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

61. A transgenic plant of claim 1, said transgenic plant all of whose cells contain at least two nucleotide sequences, each said nucleotide sequence being introduced into said transgenic plant, or an ancestor of said transgenic plant, said first introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to PKR, and said second introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to 2-5A synthetase.

62. A transgenic plant of claim 61, said transgenic plant being a transgenic tobacco plant.

63. A transgenic plant of claim 61, said first nucleotide sequence includes nucleotides designated as 1-2562 in FIG. 18 or any part of said nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR and said second nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase.

64. A transgenic plant of claim 61, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

65. A transgenic plant of claim 63, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

66. A transgenic plant of claim 1, said transgenic plant all of whose cells contain at least two nucleotide sequences, each said nucleotide sequence being introduced into said transgenic plant, or an ancestor of said transgenic plant, said first introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to 2-5A-dependent RNase and said second introduced nucleotide sequence encoding an amino acid sequence having activity similar or identical to PKR.

67. A transgenic plant of claim 66, said transgenic plant being a transgenic tobacco plant.

68. A transgenic plant of claim 66, said first nucleotide sequence includes nucleotides designated as 1-2223 in Table 1 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase.

69. A transgenic plant of claim 68, said second nucleotide sequence including nucleotides designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

70. A transgenic plant of claim 66, said first nucleotide sequence includes nucleotides selected from a group consisting of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, 1-1028 and 1-884 in Table 2.

71. A transgenic plant of claim 70, said second nucleotide sequence includes nucleotides designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

72. A transgenic plant of claim 66, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

73. A transgenic plant of claim 68, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

74. A transgenic plant of claim 69, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

75. A transgenic plant of claim 70, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

76. A transgenic plant of claim 71, said transgenic plant being selected from the group of transgenic plants consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

77. A plant transformation vector which comprises a nucleotide sequence which encodes an amino acid sequence having activity similar or identical to 2-5A-dependent RNase.

78. A plant transformation vector of claim 77, said nucleotide sequence includes nucleotides designated as 1-2223 in Table 1 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-dependent RNase.

79. A plant transformation vector of claim 77, said nucleotide sequence includes nucleotides selected from the group consisting of nucleotides designated as 1-2037, 1-1968, 1-1858, 1-1546, 1-1422, 1-1210, 1-1095, q-1028 and 1-884 in Table 2.

80. A plant transformation vector of claim 77, said vector being plasmid pAM943:2-5A-dep. RNA sense.

81. A plant cell containing said plant transformation vector of claim 77.

82. A plant cell of claim 81, said plant transformation vector being plasmid pAM943:2-5A-dep. RNase sense.

83. A plant cell of claim 81, said plant cell being a tobacco plant cell.

84. A differentiated tobacco plant comprising said tobacco plant cell of claim 83.

85. A differentiated tobacco plant of claim 84, said plant transformation vector being plasmid pAM943:2-5A-dep. RNase sense.

86. A plant cell of claim 81, said plant cell being selected from the group consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub plant cells.

87. A bacterial cell containing said plant transformation vector of claim 77.

88. A bacterial cell of claim 87, said bacterial cell being an Argobacterium tumefaciens bacterial cell.

89. A plant transformation vector which comprises a nucleotide sequence which encodes an amino acid sequence having activity similar or identical to PKR.

90. A plant transformation vector of claim 89, said nucleotide sequence includes nucleotides designated as 1-2562 in FIG. 18 or any part of this nucleotide sequence which contains the complete or partial coding sequence for PKR or the double stranded RNA binding domain of PKR.

91. A plant transformation vector of claim 89, said vector being plasmid pAM943:PK68.

92. A plant cell containing said plant transformation vector of claim 89.

93. A plant cell of claim 92, said plant cell being a tobacco plant cell.

94. A tobacco plant comprising said tobacco plant cell of claim 93.

95. A tobacco plant of claim 94, said plant transformation vector being plasmid pAM943:PK68.

96. A plant cell of claim 92, said plant cell being selected from the group consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub plant cells.

97. A plant transformation vector which comprises a nucleotide sequence which encodes an amino acid sequence having activity similar or identical to 2-5A synthetase.

98. A plant transformation vector of claim 97, said nucleotide sequence includes nucleotides designated as 1-1650 in FIG. 20 or any part of this nucleotide sequence which contains the complete or partial coding sequence for 2-5A-synthetase or the double stranded RNA binding domain of 2-5A-synthetase.

99. A plant transformation vector of claim 97, said vector being plasmid pAM943:2-5A synthetase.

100. A plant cell containing said plant transformation vector of claim 97.

101. A plant cell of claim 100, said plant cell being a tobacco plant cell.

102. A plant cell of claim 100, said plant cell being selected from the group consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub plant cells.

103. A tobacco plant comprising said tobacco plant cell of claim 101.

104. A tobacco plant of claim 94, said plant transformation vector being plasmid pAM943:synthetase.

105. A bacterial cell containing said plant transformation vector of claim 97.

106. A bacterial cell of claim 105, said bacterial cell being an Argobacterium tumefaciens bacterial cell.

107. A plant cell of claim 81, said plant cell containing a second plant transformation vector which comprises a nucleotide sequence which encodes an amino acid sequence having activity similar or identical to 2-5A synthetase.

108. A plant cell of claim 107, said plant cell containing a third plant transformation vector which comprises a nucleotide sequence which encodes an amino acid sequence having activity similar or identical to PKR.

109. A plant cell of claim 107, said plant cell being a tobacco plant cell.

110. A plant cell of claim 108, said plant cell being a tobacco plant cell.

111. A plant cell of claim 107, said plant cell being selected from the group consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub plant cells.

112. A plant cell of claim 108, said plant cell being selected from the group consisting of vegetable, fruit, grain tree, flower, grass, weed and shrub plant cells.

113. A bacterial cell containing said plant transformation vector and said second plant transformation vector of claim 107.

114. A bacterial cell of claim 113, said bacterial cell being an Argobacterium tumefaciens bacterial cell.

115. A bacterial cell containing said plant transformation vector, said second plant transformation vector and said third plant transformation vector of claim 108.

116. A bacterial cell of claim 114, said bacterial cell being an Argobacterium tumefaciens bacterial cell.

117. A transgenic plant comprising said tobacco plant cell of claim 109.

118. A transgenic plant comprising said tobacco plant cell of claim 110.

119. A transgenic plant comprising said plant cell of claim 31.

120. A transgenic plant comprising said plant cell of claim 109.

121. A transgenic plant comprising said plant cell of claim 110.

122. A transgenic plant comprising said plant cell of claim 111.

123. A transgenic plant comprising said plant cell of claim 112.

124. A method for producing genetically transformed plants which are resistant or immune to infection by a virus, said method comprises the steps of:

a.) inserting into the genome of a plant cell of a plant susceptible to a virus a construct having a nucleotide sequence which encodes for an amino acid sequence having activity similar or identical to 2-5A-dependent RNase;

b.) obtaining a transformed plant cell; and

c.) regenerating from the transformed plant cell a genetically transformed plant which expresses the amino acid sequence encoded by the construct.

125. A method of claim 124, said method including the further step of inserting into said genome of said plant cell a second construct having a nucleotide sequence which encodes for an amino acid sequence having activity similar or identical to 2-5A synthetase.

126. A method of claim 125, said method including the further step of inserting into said genome of said plant cell a second construct having a nucleotide sequence which encodes for an amino acid sequence having activity similar or identical to 2-5A synthetase.

127. A method of claim 124, said method including the further step of inserting into said genome of said plant cell a second construct having a nucleotide sequence which encodes for an amino acid sequence having activity similar or identical to PKR.

128. A method for producing genetically transformed plants which are resistant or immune to infection by a virus, said method comprises the steps of:

a.) inserting into the genome of a plant cell of a plant susceptible to a virus a construct having a nucleotide sequence which encodes for an amino acid sequence having activity similar or identical to PKR;

b.) obtaining a transformed plant cell; and

c.) regenerating from the transformed plant cell a genetically transformed plant which expresses the amino acid sequence encoded by the construct.

129. A method of claim 128, said method including the further step of inserting into said genome of said plant cell a second construct having a nucleotide sequence which encodes for an amino acid sequence having activity similar or identical to 2-5A synthetase.

130. A method for producing genetically transformed plants which are resistant or immune to infection by a virus, said method comprises the steps of:

a.) inserting into the genome of a plant cell of a plant susceptible to a virus a construct having a nucleotide sequence which encodes for an amino acid sequence having activity similar or identical to 2-5A synthetase;

b.) obtaining a transformed plant cell; and

c.) regenerating from the transformed plant cell a genetically transformed plant which expresses the amino acid sequence encoded by the construct.

131. A method of claim 124 in which the plant is a tobacco plant.

132. A method of claim 125 in which the plant is a tobacco plant.

133. A method of claim 126 in which the plant is a tobacco plant.

134. A method of claim 127 in which the plant is a tobacco plant.

135. A method of claim 128 in which the plant is a tobacco plant.

136. A method of claim 129 in which the plant is a tobacco plant.

137. A method of claim 130 in which the plant is a tobacco plant.

138. A method of claim 124 in which the plant is selected from the group consisting of vegetable, fruit, grain, flower, tree, grass, weed and shrub plants.

139. A method of claim 125 in which the plant is selected from the group consisting of vegetable, fruit, grain, flower, tree, grass, weed and shrub plants.

140. A method of claim 126 in which the plant is selected from the group consisting of vegetable, fruit, grain, flower, tree, grass, weed and shrub plants.

141. A method of claim 127 in which the plant is selected from the group consisting of vegetable, fruit, grain, flower, tree, grass, weed and shrub plants.

142. A method of claim 128 in which the plant is selected from the group consisting of vegetable, fruit, grain, flower, tree, grass, weed and shrub plants.

143. A method of claim 129 in which the plant is selected from the group consisting of vegetable, fruit, grain, flower, tree, grass, weed and shrub plants.

144. A method of claim 130 in which the plant is selected from the group consisting of vegetable, fruit, grain, flower, tree, grass, weed and shrub plants.

145. A method for producing genetically transformed plants, which are resistant or immune to infection by a virus, said method comprises the steps of:

a.) inserting into the genome of a plant cell of a plant susceptible to a virus a nucleotide sequence which encodes for an amino acid sequence having the ability to inhibit or interfere with viral replication;

b.) obtaining a transformed plant cell; and

c.) regenerating from the transformed plant cell a genetically transformed plant which expresses the amino acid sequence encoded by the nucleotide sequence.

146. A method of claim 145, the amino acid sequence having activity similar or identical to 2-5A-dependent RNase.

147. A method of claim 145, the amino acid sequence having activity similar or identical to 2-5A-synthetase.

148. A method of claim 145, the amino acid sequence having activity similar or identical to PKR.

149. A transgenic plant all of whose cells contain a nucleotide sequence introduced into said transgenic plant, or an ancestor of said transgenic plant, said introduced nucleotide sequence encoding an antisense 2-5A-dependent RNase amino acid sequence.

150. A plant transformation vector which comprises said nucleotide sequence of claim 149.

151. A plant transformation vector of claim 150, said plant transformation vector being plasmid pAM943:2-5A-dep. RNase antisense.

152. A plant transformation vector of claim 150, said plant transformation vector being plasmid pAM822:2-5A-dep. RNase antisense.

153. A construct which comprises said nucleotide sequence of claim 149, said construct being the construct as described in FIG. 13 D/a.

154. A construct which comprises said nucleotide sequence of claim 149, said construct being the construct as described in FIG. 13E.

155. A plant cell containing said plant transformation vector of claim 150.

156. A plant cell of claim 155, said plant cell being a tobacco plant cell.

157. A plant cell of claim 155, said plant cell being selected from the group consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub plant cells.

158. A bacterial cell containing said plant transformation vector of claim 150.

159. A bacterial cell of claim 158, said bacterial cell being an Argobacterium tumefaciens bacterial cell.

160. A transgenic plant of claim 149, said transgenic plant being a tobacco plant.

161. A transgenic plant of claim 149, said transgenic plant being selected from a group consisting of vegetable, fruit, grain, tree, flower, grass, weed and shrub transgenic plants.

162. An isolated nucleotide sequence encoding an amino acid sequence having human 2-5A-dependent RNAse activity, or an active fragment or analog thereof, said nucleotide sequence being identified as SEQ ID NO:3: and comprising:

ATG GAG AGC AGG GAT CAT AAC AAC CCC CAG GAG GGA CCC ACG TCC 45
TCC AGC GGT AGA AGG GCT GCA GTG GAA GAC AAT CAC TTG CTG ATT 90
AAA GCT GTT CAA AAC GAA GAT GTT GAC CTG GTC CAG CAA TTG CTG 135
GAA GGT GGA GCC AAT GTT AAT TTC CAG GAA GAG GAA GGG GGC TGG 180
ACA CCT CTG CAT AAC GCA GTA CAA ATG AGC AGG GAG GAC ATT GTG 225
GAA CTT CTG CTT CGT CAT GGT GCT GAC CCT GTT CTG AGG AAG AAG 270
AAT GGG GCC ACG CCT TTT ATC CTC GCA GCG ATT GCG GGG AGC GTG 315
AAG CTG CTG AAA CTT TTC CTT TCT AAA GGA GCA GAT GTC AAT GAG 360
TGT GAT TTT TAT GGC TTC ACA GCC TTC ATG GAA GCC GCT GTG TAT 405
GGT AAG GTC AAA GCC CTA AAA TTC CTT TAT AAG AGA GGA GCA AAT 450
GTG AAT TTG AGG CGA AAG ACA AAG GAG GAT CAA GAG CGG CTG AGG 495
AAA GGA GGG GCC ACA GCT CTC ATG GAC GCT GCT GAA AAA GGA CAC 540
GTA GAG GTC TTG AAG ATT CTC CTT GAT GAG ATG GGG GCA GAT GTA 585
AAC GCC TGT GAC AAT ATG GGC AGA AAT GCC TTG ATC CAT GCT CTC 630
CTG AGC TCT GAC GAT AGT GAT GTG GAG GCT ATT ACG CAT CTG CTG 675
CTG GAC CAT GGG GCT GAT GTC AAT GTG AGG GGA GAA AGA GGG AAG 720
ACT CCC CTG ATC CTG GCA GTG GAG AAG AAG CAC TTG GGT TTG GTG 765
CAG AGG CTT CTG GAG CAA GAG CAC ATA GAG ATT AAT GAC ACA GAC 810
AGT GAT GGC AAA ACA GCA CTG CTG CTT GCT GTT GAA CTC AAA CTG 855
AAG AAA ATC GCC GAG TTG CTG TGC AAA CGT GGA GCC AGT ACA GAT 900
TGT GGG GAT CTT GTT ATG ACA GCG AGG CGG AAT TAT GAC CAT TCC 945
CTT GTG AAG GTT CTT CTC TCT CAT GGA GCC AAA GAA GAT TTT CAC 990
CCT CCT GCT GAA GAC TGG AAG CCT CAG AGC TCA CAC TGG GGG GCA 1035
GCC CTG AAG GAT CTC CAC AGA ATA TAC CGC CCT ATG ATT GGC AAA 1080
CTC AAG TTC TTT ATT GAT GAA AAA TAC AAA ATT GCT GAT ACT TCA 1125
GAA GGA GGC ATC TAC CTG GGG TTC TAT GAG AAG CAA GAA GTA GCT 1170
GTG AAG ACG TTC TGT GAG GGC AGC CCA CGT GCA CAG CGG GAA GTC 1215
TCT TGT CTG CAA AGC AGC CGA GAG AAC AGT CAC TTG GTG ACA TTC 1260
TAT GGG AGT GAG AGC CAC AGG GGC CAC TTG TTT GTG TGT GTC ACC 1305
CTC TGT GAG CAG ACT CTG GAA GCG TGT TTG GAT GTG CAC AGA GGG 1350
GAA GAT GTG GAA AAT GAG GAA GAT GAA TTT GCC CGA AAT GTC CTG 1395
TCA TCT ATA TTT AAG GCT GTT CAA GAA CTA CAC TTG TCC TGT GGA 1440
TAC ACC CAC CAG GAT CTG CAA CCA CAA AAC ATC TTA ATA GAT TCT 1485
AAG AAA GCT GCT CAC CTG GCA GAT TTT GAT AAG AGC ATC AAG TGG 1530
GCT GGA GAT CCA CAG GAA GTC AAG AGA GAT CTA GAG GAC CTT GGA 1575
CGG CTG GTC CTC TAT GTG GTA AAG AAG GGA AGC ATC TCA TTT GAG 1620

GAT CTG AAA GCT CAA AGT AAT GAA GAG GTG GTT CAA CTT TCT CCA 1665
GAT GAG GAA ACT AAG GAC CTC ATT CAT CGT CTC TTC CAT CCT GGG 1710
GAA CAT GTG AGG GAC TGT CTG AGT GAC CTG CTG GGT CAT CCC TTC 1755
TTT TGG ACT TGG GAG AGC CGC TAT AGG ACG CTT CGG AAT GTG GGA 1800
AAT GAA TCC GAC ATC AAA ACA CGA AAA TCT GAA AGT GAG ATC CTC 1845
AGA CTA CTG CAA CCT GGG CCT TCT GAA CAT TCC AAA AGT TTT GAC 1890
AAG TGG ACG ACT AAG ATT AAT GAA TGT GTT ATG AAA AAA ATG AAT 1935
AAG TTT TAT GAA AAA AGA GGC AAT TTC TAC CAG AAC ACT GTG GGT 1980
GAT CTG CTA AAG TTC ATC CGG AAT TTG GGA GAA CAC ATT GAT GAA 2025
GAA AAG CAT AAA AAG ATG AAA TTA AAA ATT GGA GAC CCT TCC CTG 2070
TAT TTT CAG AAG ACA TTT CCA GAT CTG GTG ATC TAT GTC TAC ACA 2115
AAA CTA CAG AAC ACA GAA TAT AGA AAG CAT TTC CCC CAA ACC CAC 2160
AGT CCA AAC AAA CCT CAG TGT GAT GGA GCT GGT GGG GCC AGT GGG 2205
TTG GCC AGC CCT GGG TGC 2223

163. An amino acid sequence having human 2-5A-dependent RNAse activity, or an active fragment or analog thereof, said amino acid sequence being identified as SEQ ID NO:4: and comprising:

Met Glu Ser Arg Asp His Asn Asn Pro Gln Glu Gly Pro Thr Ser 15
Ser Ser Gly Arg Arg Ala Ala Val Glu Asp Asn His Leu Leu Ile 30
Lys Ala Val Gln Asn Glu Asp Val Asp Leu Val Gln Gln Leu Leu 45
Glu Gly Gly Ala Asn Val Asn Phe Gln Glu Glu Glu Gly Gly Trp 60
Thr Pro Leu His Asn Ala Val Gln Met Ser Arg Glu Asp Ile Val 75
Glu Leu Leu Leu Arg His Gly Ala Asp Pro Val Leu Arg Lys Lys 90
Asn Gly Ala Thr Pro Phe Ile Leu Ala Ala Ile Ala Gly Ser Val 105
Lys Leu Leu Lys Leu Phe Leu Ser Lys Gly Ala Asp Val Asn Glu 120
Cys Asp Phe Tyr Gly Phe Thr Ala Phe Met Glu Ala Ala Val Tyr 135
Gly Lys Val Lys Ala Leu Lys Phe Leu Tyr Lys Arg Gly Ala Asn 150
Val Asn Leu Arg Arg Lys Thr Lys Glu Asp Gln Glu Arg Leu Arg 165
Lys Gly Gly Ala Thr Ala Leu Met Asp Ala Ala Glu Lys Gly His 180
Val Glu Val Leu Lys Ile Leu Leu Asp Glu Met Gly Ala Asp Val 195
Asn Ala Cys Asp Asn Met Gly Arg Asn Ala Leu Ile His Ala Leu 210
Leu Ser Ser Asp Asp Ser Asp Val Glu Ala Ile Thr His Leu Leu 225
Leu Asp His Gly Ala Asp Val Asn Val Arg Gly Glu Arg Gly Lys 240
Thr Pro Leu Ile Leu Ala Val Glu Lys Lys His Leu Gly Leu Val 255
Gln Arg Leu Leu Glu Gln Glu His Ile Glu Ile Asn Asp Thr Asp 270
Ser Asp Gly Lys Thr Ala Leu Leu Leu Ala Val Glu Leu Lys Leu 285
Lys Lys Ile Ala Glu Leu Leu Cys Lys Arg Gly Ala Ser Thr Asp 300
Cys Gly Asp Leu Val Met Thr Ala Arg Arg Asn Tyr Asp His Ser 315
Leu Val Lys Val Leu Leu Ser His Gly Ala Lys Glu Asp Phe His 330
Pro Pro Ala Glu Asp Trp Lys Pro Gln Ser Ser His Trp Gly Ala 345
Ala Leu Lys Asp Leu His Arg Ile Tyr Arg Pro Met Ile Gly Lys 360
Leu Lys Phe Phe Ile Asp Glu Lys Tyr Lys Ile Ala Asp Thr Ser 375
Glu Gly Gly Ile Tyr Leu Gly Phe Tyr Glu Lys Gln Glu Val Ala 390
Val Lys Thr Phe Cys Glu Gly Ser Pro Arg Ala Gln Arg Glu Val 405
Ser Cys Leu Gln Ser Ser Arg Glu Asn Ser His Leu Val Thr Phe 420
Tyr Gly Ser Glu Ser His Arg Gly His Leu Phe Val Cys Val Thr 435
Leu Cys Glu Gln Thr Leu Glu Ala Cys Leu Asp Val His Arg Gly 450
Glu Asp Val Glu Asn Glu Glu Asp Glu Phe Ala Arg Asn Val Leu 465
Ser Ser Ile Phe Lys Ala Val Gln Glu Leu His Leu Ser Cys Gly 480
Tyr Thr His Gln Asp Leu Gln Pro Gln Asn Ile Leu Ile Asp Ser 495
Lys Lys Ala Ala His Leu Ala Asp Phe Asp Lys Ser Ile Lys Trp 510
Ala Gly Asp Pro Gln Glu Val Lys Arg Asp Leu Glu Asp Leu Gly 525
Arg Leu Val Leu Tyr Val Val Lys Lys Gly Ser Ile Ser Phe Glu 540
Asp Leu Lys Ala Gln Ser Asn Glu Glu Val Val Gln Leu Ser Pro 555

Asp Glu Glu Thr Lys Asp Leu Ile His Arg Leu Phe His Pro Gly 570
Glu His Val Arg Asp Cys Leu Ser Asp Leu Leu Gly His Pro Phe 585
Phe Trp Thr Trp Glu Ser Arg Tyr Arg Thr Leu Arg Asn Val Gly 600
Asn Glu Ser Asp Ile Lys Thr Arg Lys Ser Glu Ser Glu Ile Leu 615
Arg Leu Leu Gln Pro Gly Pro Ser Glu His Ser Lys Ser Phe Asp 630
Lys Trp Thr Thr Lys Ile Asn Glu Cys Val Met Lys Lys Met Asn 645
Lys Phe Tyr Glu Lys Arg Gly Asn Phe Tyr Gln Asn Thr Val Gly 660
Asp Leu Leu Lys Phe Ile Arg Asn Leu Gly Glu His Ile Asp Glu 675
Glu Lys His Lys Lys Met Lys Leu Lys Ile Gly Asp Pro Ser Leu 690
Tyr Phe Gln Lys Thr Phe Pro Asp Leu Val Ile Tyr Val Tyr Thr 705
Lys Leu Gln Asn Thr Glu Tyr Arg Lys His Phe Pro Gln Thr His 720
Ser Pro Asn Lys Pro Gln Cys Asp Gly Ala Gly Gly Ala Ser Gly 735
Leu Ala Ser Pro Gly Cys 741

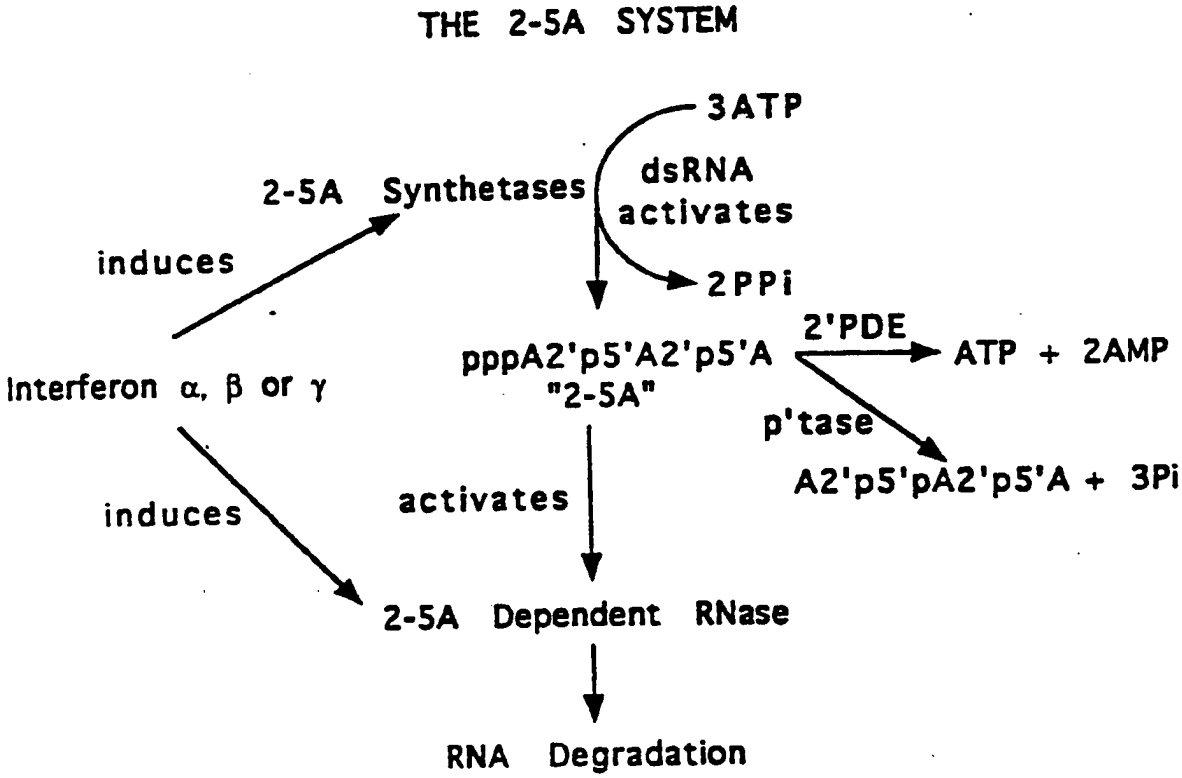

THE 2-5A SYSTEM
3ATP
dsRNA
activates
2-5A Synthetases
induces
2PPi
2'PDE
pppA2'p5'A2'p5'A
"2-5A"
ATP + 2AMP
Interferon α, β or γ
p'tase
A2'p5'pA2'p5'A + 3Pi
induces
activates
2-5A Dependent RNase
RNA Degradation

FIG. 1

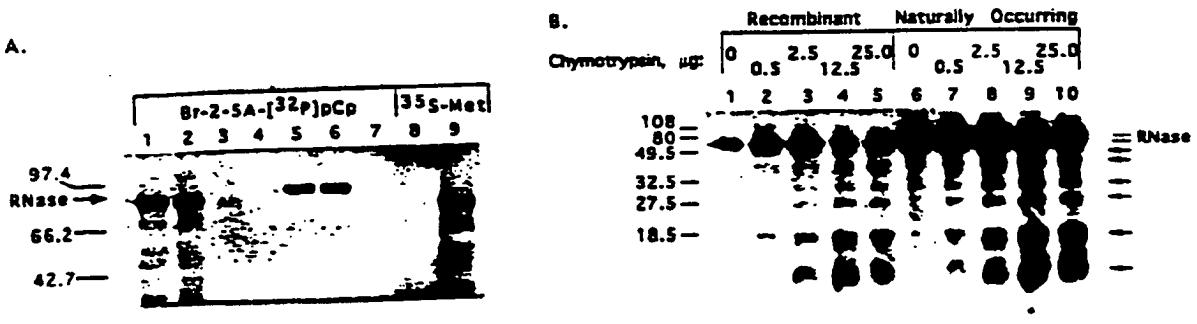

A.
Br-2-5A-[32P]pCp
35S-Met
1 2 3 4 5 6 7 8 9
97.4
RNase
66.2
42.7
B.
Recombinant
Naturally Occurring
Chymotrypsin, µg:
0 0.5 2.5 12.5 25.0 0 0.5 2.5 12.5 25.0
1 2 3 4 5 6 7 8 9 10
108
80
49.5
32.5
27.5
18.5
RNase

FIG. 2

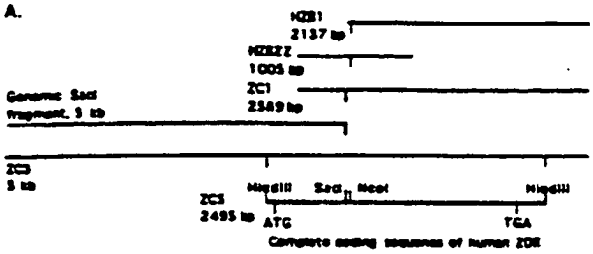

A.
2137 bp
1005 bp
2569 bp
Genomic SacI fragment, 5 kb
3 kb
2495 bp
HindIII
SacI
NcoI
HindIII
ATG
TGA

FIG. 3

P-loop motifs- ■ Cys-rich- ▨ PK homology- □

```
Human  -  MESRDHNNPQ EGPTSSSGRR AAVEDNHLLI KAVQNEDVDL VQQLLEGGAN VNFQEEEGGW   60
Murine -  METPDYNTPQ GGTPSAGSQR TVVEDDSSLI KAVQKGDVVR VQQLLEKGAD ANACEDTWGW   60

Human  -  TPLHNAVQMS REDIVELLLR HGADPVLRKK NGATLFILAA IAGSVKLLKL FLSKGADVNE  120
Murine -  TPLHNAVQAG RVDIVNLLLS HGADPHRRKK NGATPFIIAG IQGDVKLLEI LLSCGADVNE  120

Human  -  CDFYGFTAFM EAAVYGKVKA LKFLYKRGAN VNLRRKTKED QERLRKGGAT ALMDAAEKGH  180
Murine -  CDENGFTAFM EAAERGNAEA LRFLFAKGAN VNLRRQTTKD KRRLKQGGAT ALMSAAEKGH  180

Human  -  VEVLKILLDE MGADVNACDN MGRNALIHAL LSSDDSDVEA ITHLLLDHGA DVNVRGERGK  240
Murine -  LEVLRILLND MKAEVDARDN MGRNALIRTL LNWDCENVEE ITSILIQHGA DVNVRGERGK  240

Human  -  TPLILAVEKK HLGLVQRLLE QEHIEINDTD SDGKTALLLA VELKLKKIAE LLCKRGASTD  300
Murine -  TPLIAAVERK HTGLVQMLLS REGINIDARD NEGKTALLIA VDKQLKEIVQ LLLEKGA-DK  299

Human  -  CGDLVMTARR NYDHSLVKVL LSHGAKEDFH PPAEDWKPQS SHWGAALKDL HRIYRPMIGK  360
Murine -  CDDLVWIARR NHDYHLVKLL LPYVANPDTD PPAGDWSPHS SRWGTALKSL HSMTRPMIGK  359

Human  -  LKFFIDEKYK IADTSEGGIY LGFYEKQEVA VKTFCEGSPR AQREVSCLQS SRENSHLVTF  420
Murine -  LKIFIHDDYK IAGTSEGAVY LGIYDNREVA VKVFRENSPR GCKEVSCLRD CGDHSNLVAF  419

Human  -  YGSESHRGHL FVCVTLCEQT LEACLDVHRG EDVENEEDEF ARNVLSSIFK AVQELHLSCG  480
Murine -  YGREDDKGCL YVCVSLCEWT LEEFLRLPRE EPVENGEDKF AHSILLSIFE GVQKLHLH-G  478

Human  -  YTHQDLQPQN ILIDSKKAAH LADFDKSIKW AGDPQEVKRD LEDLGRLVLY VVKKGSISFE  540
Murine -  YSHQDLQPQN ILIDSKKAVR LADFDQSIRW MGESQMVRRD LEDLGRLVLY VVMKGEIPFE  538

Human  -  DLKAQSNEEV VQLSPDEETK DLIHRLFHPG EHVRDCLSDL LGHPFFWTWE SRYRTLRNVG  600
Murine -  TLKTQNDEVL LTMSPDEETK DLIHCLFSPG ENVKNCLVDL LGHPFFWTWE NRYRTLRNVG  598

Human  -  NESDIKTRKS ESEILRLLQP GPSEHSKSFD KWTTKINECV MKKMNKFYEK R-GNFYQNTV  659
Murine -  NESDIKVRKC KSDLLRLLQH QTLEPPRSFD QWTSKIDKNV MDEMNHFYEK RKKNPYQDTV  658

Human  -  GDLLKFIRNL GEHIDEEKHK KMKLKIGDPS LYFQKTFPDL VIYVYTKLQN TEYRKHFPQT  719
Murine -  GDLLKFIRNI GEHINEEKKR G--------- ---------- ---------- ----------  679

Human  -  HSPNKPQCDG AGGASGLASP GC   741
```

FIG. 4

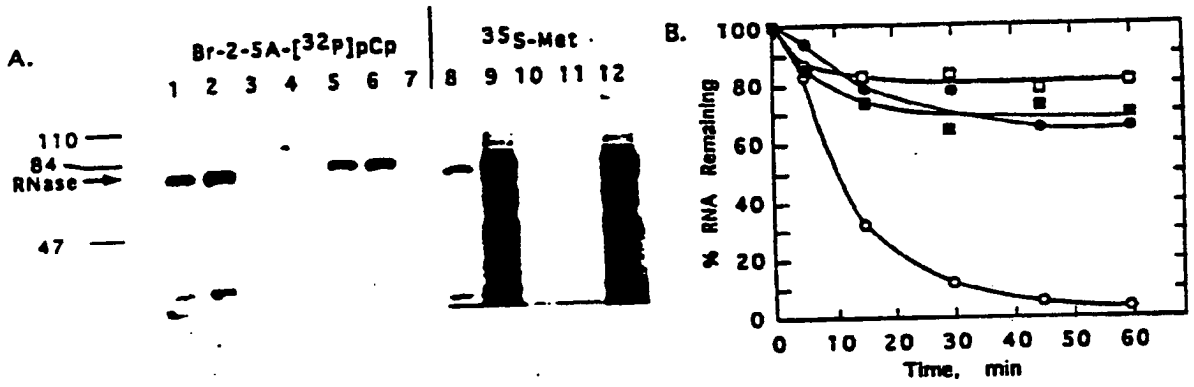

A.
Br-2-5A-[32P]pCp
35S-Met
1 2 3 4 5 6 7 8 9 10 11 12
110
84
RNase
47
B.
% RNA Remaining
100
80
60
40
20
0
0 10 20 30 40 50 60
Time, min

FIG. 5

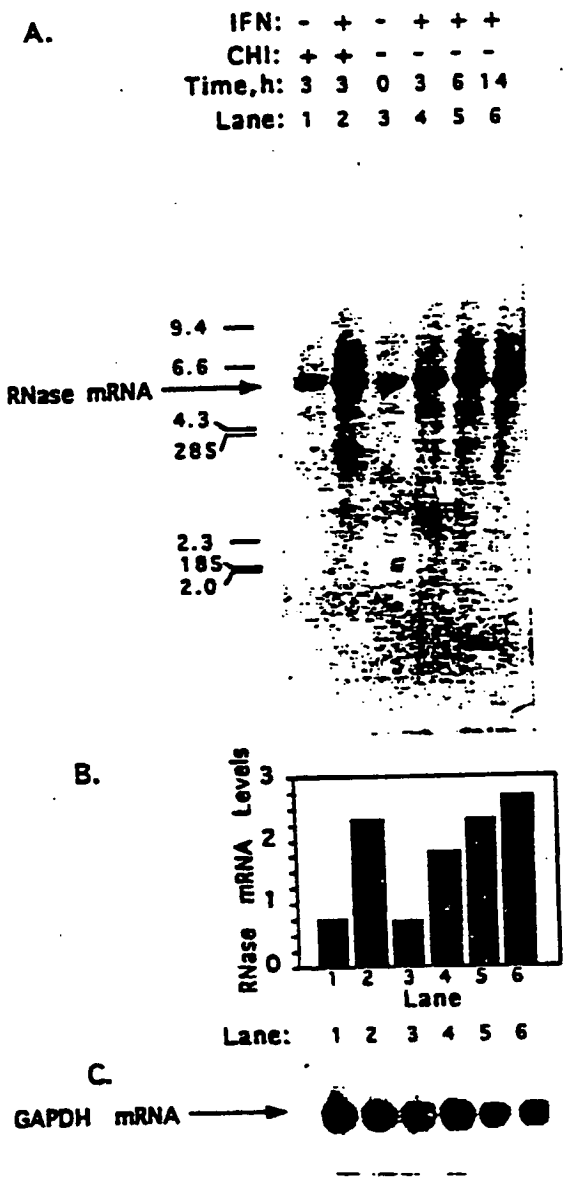

A.
IFN: - + - + + +
CHI: + + - - - -
Time,h: 3 3 0 3 6 14
Lane: 1 2 3 4 5 6
9.4
6.6
RNase mRNA
4.3
28S
2.3
18S
2.0
B.
RNase mRNA Levels
0
1
2
3
1 2 3 4 5 6
Lane
Lane: 1 2 3 4 5 6
C.
GAPDH mRNA

FIG. 6

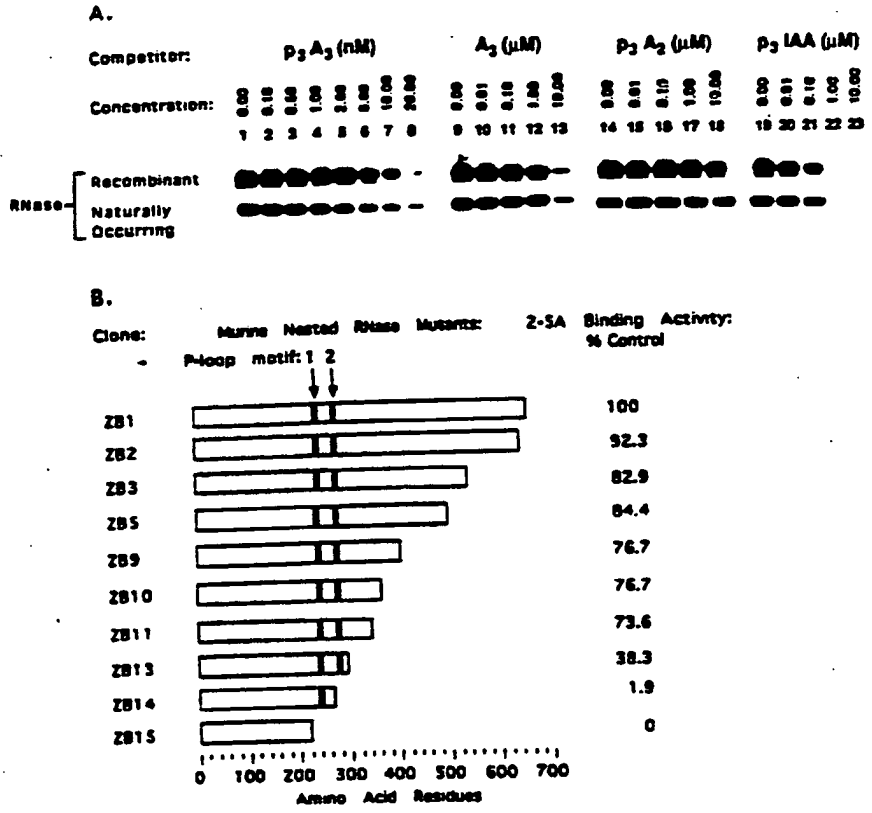

A.
Competitor:
p_3A_3 (nM)
A_3 (μM)
p_3A_2 (μM)
p_3 IAA (μM)
Concentration:
1 2 3 4 5 6 7 8
9 10 11 12 13
14 15 16 17 18
19 20 21 22 23
RNase
Recombinant
Naturally Occurring
B.
Clone:
Murine Nested RNase Mutants:
2-5A Binding Activity: % Control
P-loop motif: 1 2
ZB1
ZB2
ZB3
ZB5
ZB9
ZB10
ZB11
ZB13
ZB14
ZB15
100
92.3
82.9
84.4
76.7
76.7
73.6
38.3
1.9
0
0 100 200 300 400 500 600 700
Amino Acid Residues

FIG. 7

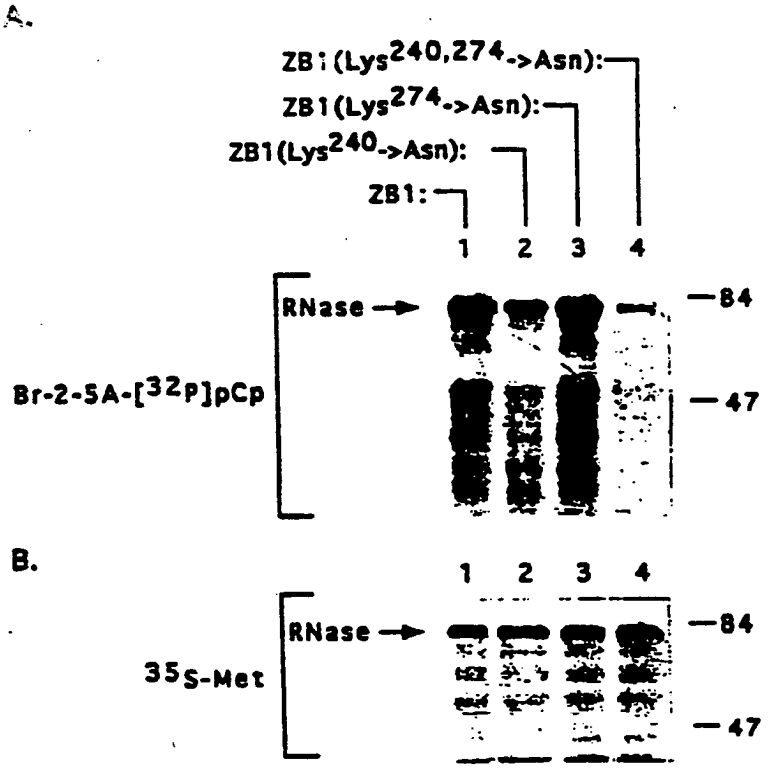

A.
ZB1 (Lys240,274->Asn):
ZB1 (Lys274->Asn):
ZB1 (Lys240->Asn):
ZB1:
1 2 3 4
RNase
Br-2-5A-[32P]pCp
84
47
B.
1 2 3 4
RNase
35S-Met
84
47

FIG. 8

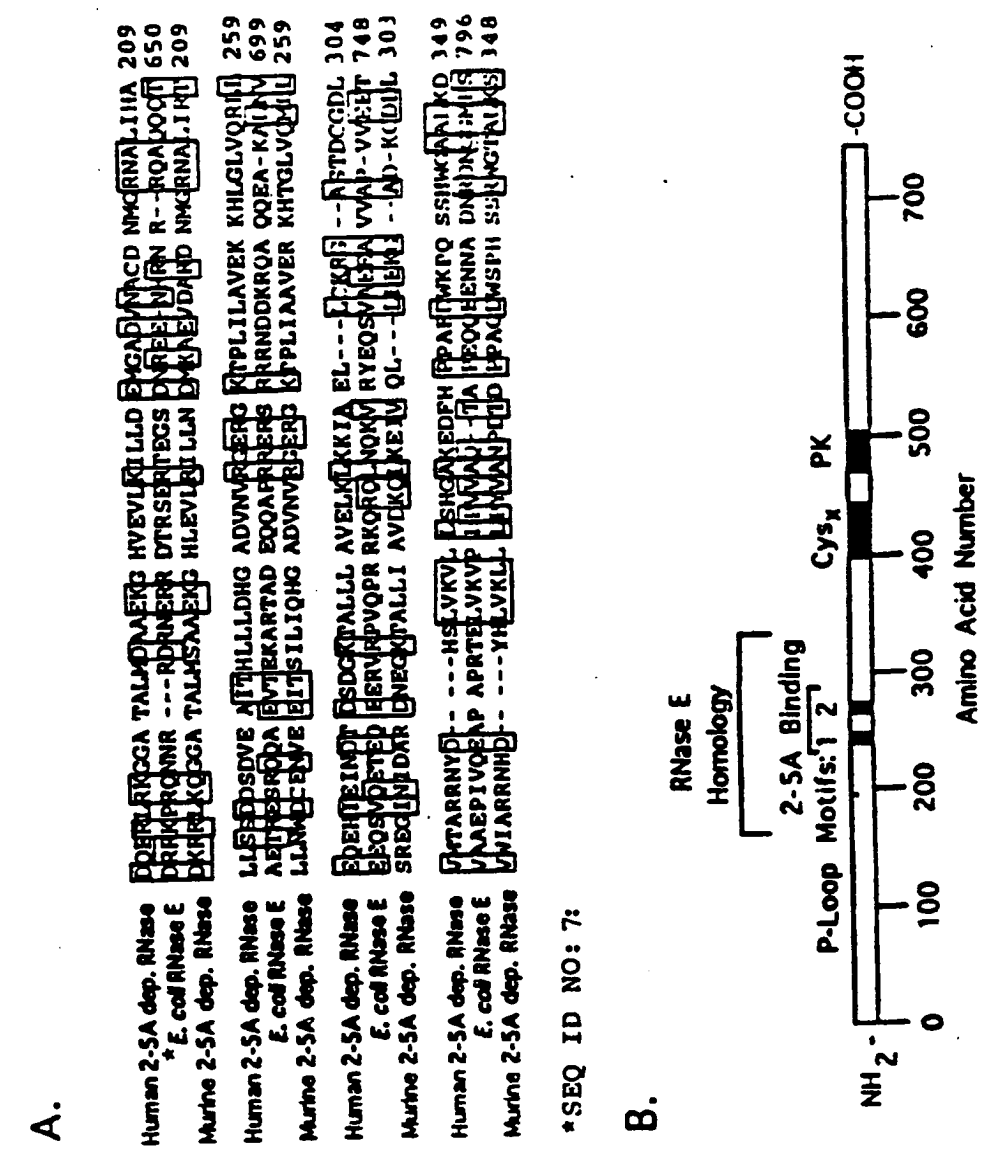
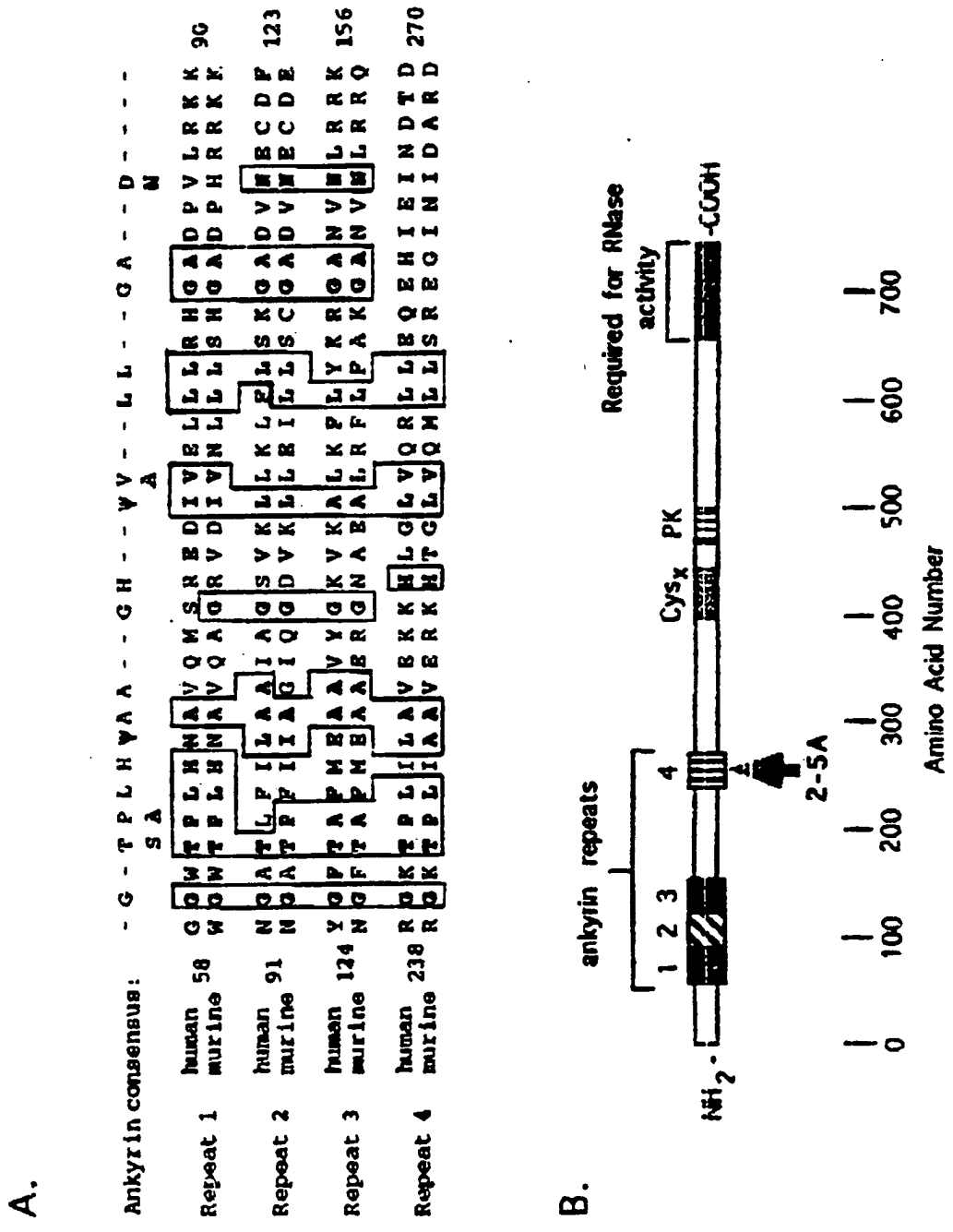

A.
Human 2-5A dep. RNase
*E. coli RNase E
Murine 2-5A dep. RNase
Human 2-5A dep. RNase
E. coli RNase E
Murine 2-5A dep. RNase
Human 2-5A dep. RNase
E. coli RNase E
Murine 2-5A dep. RNase
Human 2-5A dep. RNase
E. coli RNase E
Murine 2-5A dep. RNase
*SEQ ID NO: 7:
B.
RNase E
Homology
2-5A Binding
P-Loop Motifs: 1 2
Cys x PK
NH2
-COOH
0
100
200
300
400
500
600
700
Amino Acid Number

FIG. 9

FIG. 10


A.
Ankyrin consensus:
Repeat 1
Repeat 2
Repeat 3
Repeat 4
human
murine
B.
ankyrin repeats
1 2 3 4
Cys$_x$ PK
Required for RNase activity
-COOH
NH$_2$-
2-5A
0 100 200 300 400 500 600 700
Amino Acid Number

ROLE OF 2-5A IN THE ANTIVIRAL RESPONSE OF CELLS TO INTERFERON (IFN) TREATMENT

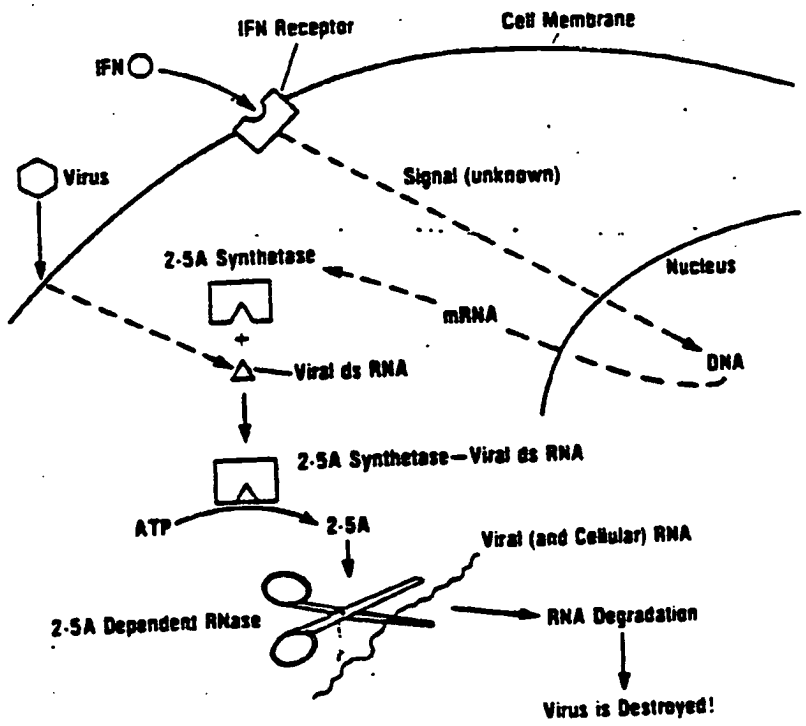


FIG. 11

FIG. 12

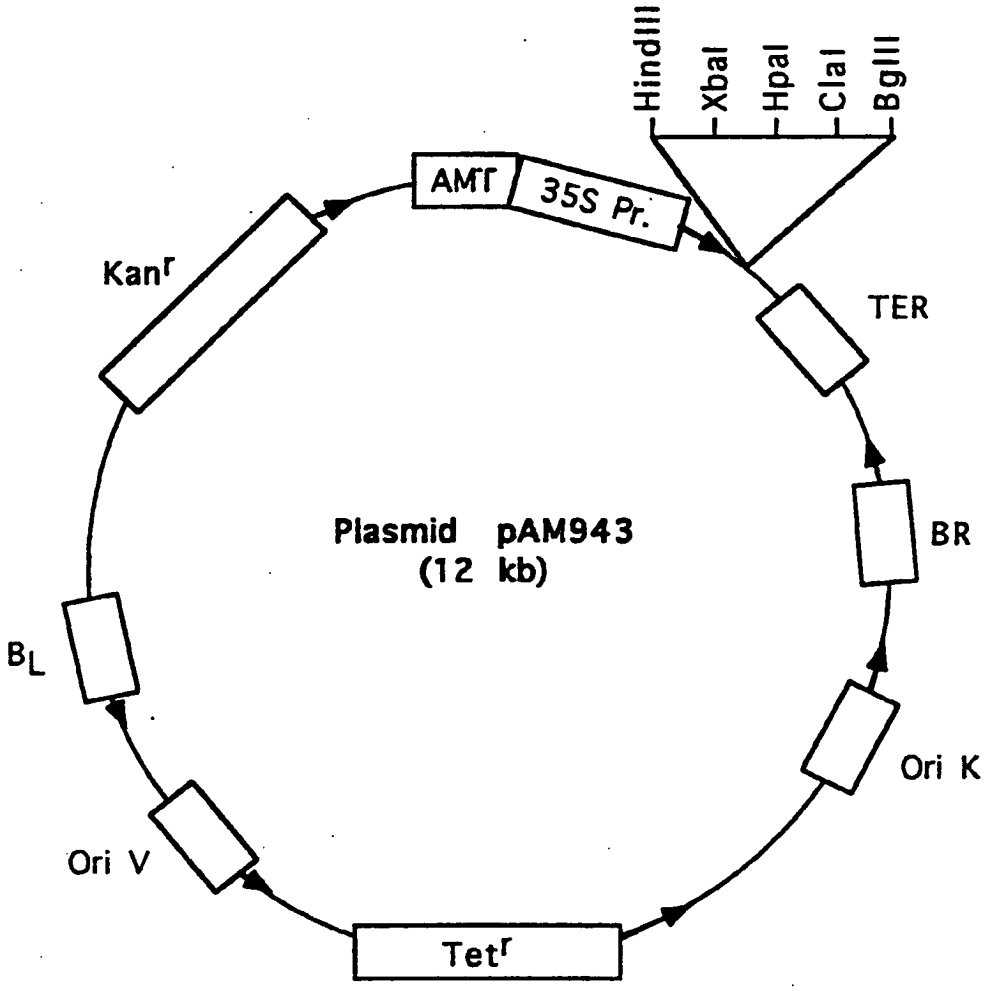

HindIII
XbaI
HpaI
ClaI
BglII
AMT
35S Pr.
Kan^r
TER
Plasmid pAM943
(12 kb)
BR
B_L
Ori K
Ori V
Tet^r

FIG. 13

Contains
Portions of Plasmid Constructs Containing cDNAs Encoding Mammalian Antiviral Proteins

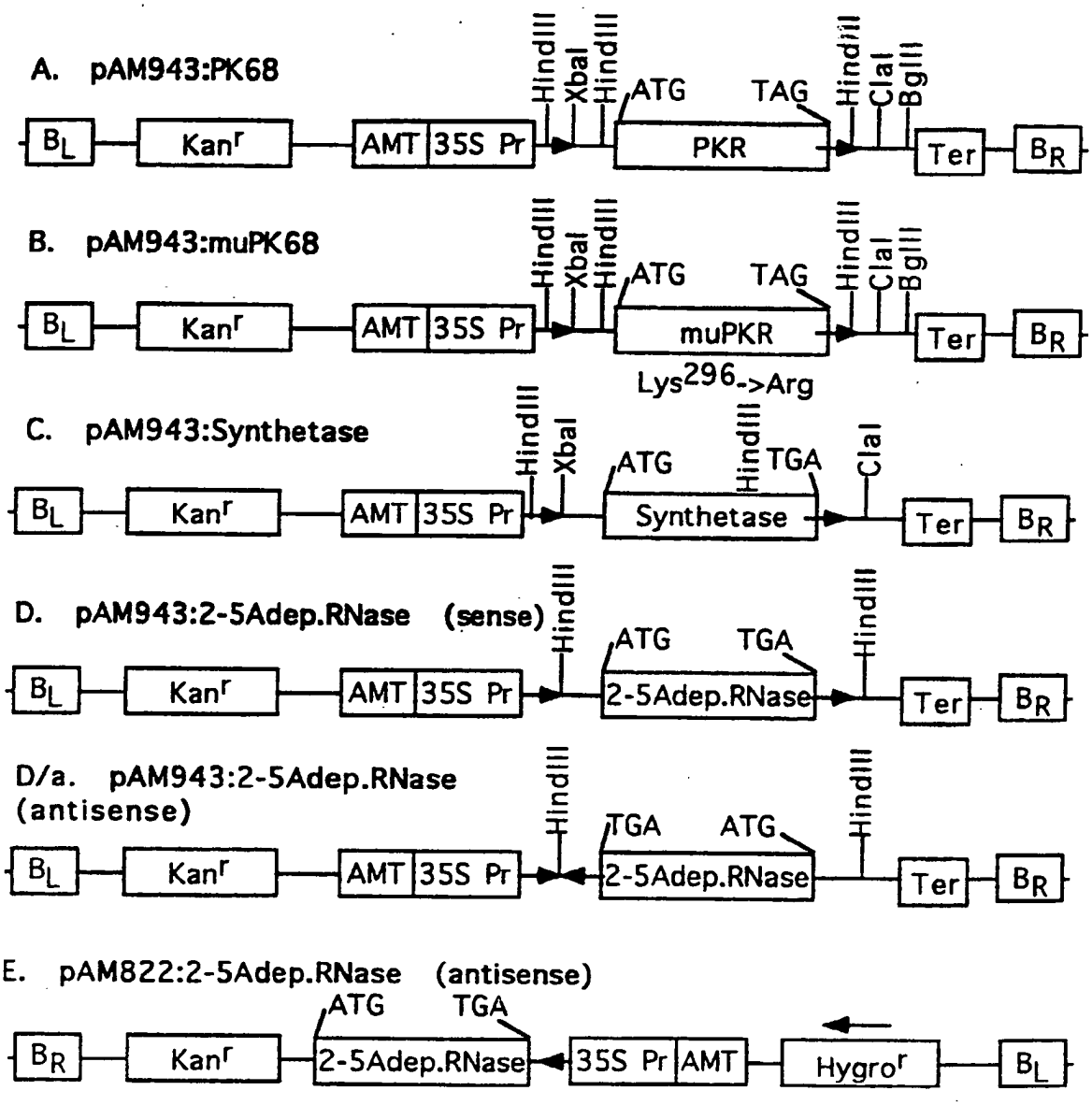


FIG. 14

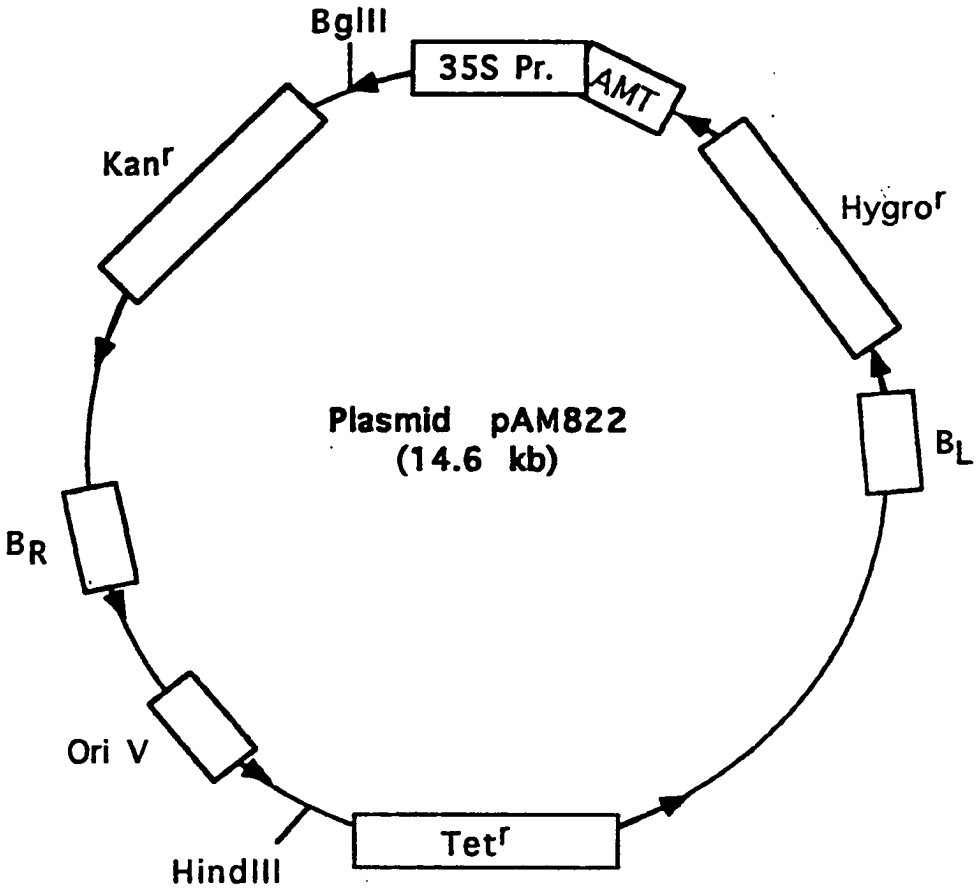

BglII
35S Pr.
AMT
Hygro^r
B_L
Plasmid pAM822
(14.6 kb)
B_R
Ori V
HindIII
Tet^r
Kan^r

FIG. 15

Expression of human 2-5A-synthetase cDNA in transgenic tobacco plants as determined by measuring mRNA levels in a Northern Blot.

	Control	2-5A-Synthetase			
Plant Number:	C	1	4	14	16 18

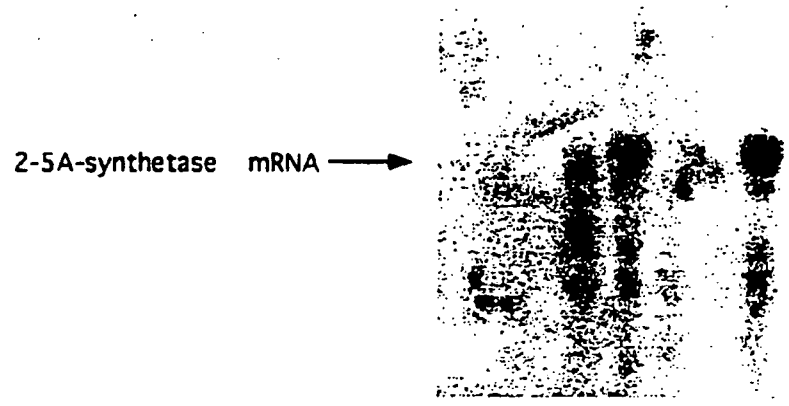


FIG. 16

Expression of mutant and wild type forms of human PKR cDNA in transgenic tobacco plants as determined by measuring mRNA levels in a Northern Blot.

	Control	Mutant PKR							Wild Type PKR			
Plant Number:	C	2	6	7	10	11	12	17	1	5	8	10

FIG. 17

Presence of 2-5A-dependent RNase cDNA in transgenic tobacco plants as determined on a Southern blot

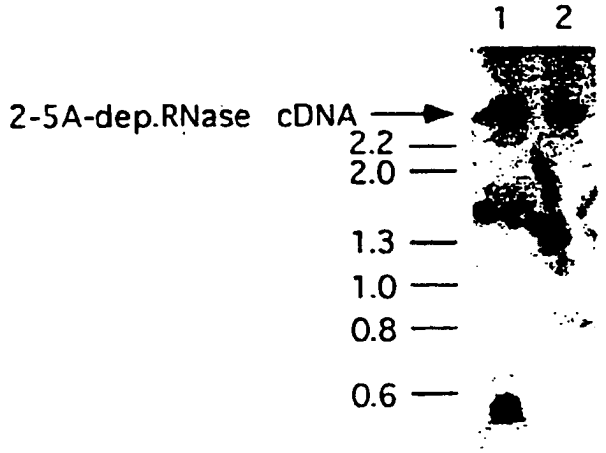


FIG. 18

Human p68 Kinase mRNA (PKR) Coding Sequence

SEQ ID NO:8:

1	cagtttctgg	agcaaattca	gtttgccttc	ctggatttgt	aaattgtaat	gacctcaaaa
61	ctttagcagt	tcttccatct	gactcaggtt	tgcttctctg	gcggtcttca	gaatcaacat
121	ccacacttcc	gtgattatct	gcgtgcattt	tggacaaagc	ttccaaccag	gatacgggaa
181	gaagaaatgg	ctggtgatct	ttcagcaggt	ttcttcatgg	aggaacttaa	tacataccgt
241	cagaagcagg	gagtagtact	taaatatcaa	gaactgccta	attcaggacc	tccacatgat
301	aggaggttta	catttcaagt	tataatagat	ggaagagaat	ttccagaagg	tgaaggtaga
361	tcaaagaagg	aagcaaaaaa	tgccgcagcc	aaattagctg	ttgagatact	taataaggaa
421	aagaaggcag	ttagtccttt	attattgaca	acaacgaatt	cttcagaagg	attatccatg
481	gggaattaca	taggccttat	caatagaatt	gcccagaaga	aaagactaac	tgtaaattat
541	gaacagtgtg	catcgggggt	gcatgggcca	gaaggatttc	attataaatg	caaaatggga
601	cagaaagaat	atagtattgg	tacaggttct	actaaacagg	aagcaaaaca	attggccgct
661	aaacttgcat	atcttcagat	attatcagaa	gaaacctcag	tgaaatctga	ctacctgtcc
721	tctggttctt	ttgctactac	gtgtgagtcc	caaagcaact	ctttagtgac	cagcacactc
781	gcttctgaat	catcatctga	aggtgacttc	tcagcagata	catcagagat	aaattctaac
841	agtgacagtt	taaacagttc	ttcgttgctt	atgaatggtc	tcagaaataa	tcaaaggaag
901	gcaaaaagat	ctttggcacc	cagatttgac	cttcctgaca	tgaaagaaac	aaagtatact
961	gtggacaaga	ggtttggcat	ggattttaaa	gaaatagaat	taattggctc	aggtggattt
1021	ggccaagttt	tcaaagcaaa	acacagaatt	gacggaaaga	cttacgttat	taaacgtgtt
1081	aaatataata	acgagaaggc	ggagcgtgaa	gtaaaagcat	tggcaaaact	tgatcatgta
1141	aatattgttc	actacaatgg	ctgttgggat	ggatttgatt	atgatcctga	gaccagtgat
1201	gattctcttg	agagcagtga	ttatgatcct	gagaacagca	aaaatagttc	aaggtcaaag
1261	actaagtgcc	ttttcatcca	aatggaattc	tgtgataaag	ggaccttgga	acaatggatt
1321	gaaaaaagaa	gaggcgagaa	actagacaaa	gttttggctt	tggaactctt	tgaacaaata
1381	acaaaagggg	tggattatat	acattcaaaa	aaattaattc	atagagatct	taagccaagt
1441	aatatattct	tagtagatac	aaaacaagta	aagattggag	actttggact	tgtaacatct
1501	ctgaaaaatg	atggaaagcg	aacaaggagt	aggggaactt	tgcgatacat	gagcccagaa
1561	cagatttctt	cgcaagacta	tggaaaggaa	gtggacctct	acgctttggg	gctaattctt
1621	gctgaacttc	ttcatgtatg	tgacactgct	tttgaaacat	caaagttttt	cacagaccta
1681	cgggatggca	tcatctcaga	tatatttgat	aaaaaagaaa	aaactcttct	acagaaatta
1741	ctctcaaaga	aacctgagga	tcgacctaac	acatctgaaa	tactaaggac	cttgactgtg
1801	tggaagaaaa	gcccagagaa	aaatgaacga	cacacatgtt	agagcccttc	tgaaaaagta
1861	tcctgcttct	gatatgcagt	tttccttaaa	ttatctaaaa	tctgctaggg	aatatcaata
1921	gatatttacc	ttttatttta	atgtttcctt	taatttttta	ctatttttac	taatctttct
1981	gcagaaacag	aaaggttttc	ttctttttgc	ttcaaaaaca	ttcttacatt	ttacttttttc
2041	ctggctcatc	tctttatttt	tttttttttt	ttttaaagac	agagtctcgc	tctgttgccc
2021	aggctggagt	gcaatgacac	agtcttggct	cactgcaact	tctgcctctt	gggttcaagt
2061	gattctcctg	cctcagcctc	ctgagtagct	ggattacagg	catgtgccac	ccacccaact
2221	aatttttgtg	tttttaataa	agacagggtt	tcaccatgtt	ggccaggctg	gtctcaaact
2281	cctgacctca	agtaatccac	ctgcctcggc	ctcccaaagt	gctgggatta	cagggatgag
2341	ccaccgcgcc	cagcctcatc	tctttgttct	aaagatggaa	aaaccacccc	caaatttttct
2401	ttttatacta	ttaatgaatc	aatcaattca	tatctattta	ttaaatttct	accgctttta
2461	ggccaaaaaa	atgtaagatc	gttctctgcc	tcacatagct	tacaagccag	ctggagaaat
2521	atggtactca	ttaaaaaaaa	aaaaaaaaag	tgatgtacaa	cc	

FIG. 19

Human PKR Amino Acid Sequence

SEQ ID NO:9:

MAGDLSAGFFMEELNTYRQKQGVVLKYQELPNSGPPHDRRFTFQVIID
GREFPEGEGRSKKEAKNAAAKLAVEILNKEKKAVSPLLLTTTNSSEGLS
MGNYIGLINRIAQKKRLTVNYEQCASGVHGPEGFHYKCKMGQKEYSIG
TGSTKQEAKQLAAKLAYLQILSEETSVKSDYLSSGSFATTCESQSNSLV
TSTLASESSSEGDFSADTSEINSNSDSLNSSSLLMNGLRNNQRKAKRS
LAPRFDLPDMKETKYTVDKRFGMDFKEIELIGSGGFGQVFKAKHRIDG
KTYVIKRVKYNNEKAEREVKALAKLDHVNIVHYNGCWDGFDYDPETSD
DSLESSDYDPENSKNSSRSKTKCLFIQMEFCDKGTLEQWIEKRRGEKL
DKVLALELFEQITKGVDYIHSKKLIHRDLKPSNIFLVDTKQVKIGDFGLVT
SLKNDGKRTRSKGTLRYMSPEQISSQDYGKEVDLYALGLILAELLHVCD
TAFETSKFFTDLRDGIISDIFDKKEKTLLQKLLSKKPEDRPNTSEILRTLT
VWKKSPEKNERHTC

FIG. 20

Human 2-5A-Synthetase cDNA

SEQ ID NO:10:

```
                10         20         30         40         50
  1 AACTGAAACC AACAGCAGTC CAAGCTCAGT CAGCAGAAGA GATAAAAGCA

                60         70         80         90        100
 51 AACAGGTCTG GGAGGCAGTT CTGTTGCCAC TCTCTCTCCT GTCAATGATG

                10         20         30         40         50
101 GATCTCAGAA ATACCCCAGC CAAATCTCTG GACAAGTTCA TTGAAGACTA

                60         70         80         90        100
151 TCTCTTGCCA GACACGTGTT TCCGCATGCA AATCGACCAT GCCATTGACA

                10         20         30         40         50
201 TCATCTGTGG GTTCCTGAAG GAAAGGTGCT TCCGAGGTAG CTCCTACCCT

                60         70         80         90        100
251 GTGTGTGTGT CCAAGGTGGT AAAGGGTGGC TCCTCAGGCA AGGGCACCAC

                10         20         30         40         50
301 CCTCAGAGGC CGATCTGACG CTGACCTGGT TGTCTTCCTC AGTCCTCTCA

                60         70         80         90        100
351 GCACTTTTCA GGATCAGTTA AATCGCCGGG GAGAGTTCAT CCAGGAAATT

                10         20         30         40         50
401 AGGAGACAGC TGGAAGCCTG TCAAAGAGAG AGAGCACTTT CCGTGAAGTT

                60         70         80         90        100
451 TGAGGTCCAG GCTCCACGCT GGGGCAACCC CCGTGCGCTC AGCTTCGTAC

                10         20         30         40         50
501 TGAGTTCGCT CCAGCTCGGG GAGGGGGTGG AGTTCGATGT GCTGCCTGCC

                60         70         80         90        100
551 TTTGATGCCC TGGGTCAGTT GACTGGCAGC TATAAACCTA ACCCCCAAAT

                10         20         30         40         50
601 CTATGTCAAG CTCATCGAGG AGTGCACCGA CCTGCAGAAA GAGGGCGAGT

                60         70         80         90        100
651 TCTCCACCTG CTTCACAGAA CTACAGAGAG ACTTCCTGAA GCAGCGCCCC

                10         20         30         40         50
701 ACCAAGCTCA AGAGCCTCAT CCGCCTAGTC AAGCACTGGT ACCAAAATTG

                60         70         80         90        100
751 TAAGAAGAAG CTTGGGAAGC TGCCACCTCA GTATGCCCTG GAGCTCCTGA

                10         20         30         40         50
801 CGGTCTATGC TTGGGAGCGA GGGAGCATGA AAACACATTT CAACACAGCC

                60         70         80         90        100
851 CAAGGATTTC GGACGGTCTT GGAATTAGTC ATAAACTACC AGCAACTCTG
```

FIG. 20 (cont.)

```
                10         20         30         40         50
901 CATCTACTGG ACAAAGTATT ATGACTTTAA AAACCCCATT ATTGAAAAGT

                60         70         80         90        100
951 ACCTGAGAAG GCAGCTCACG AAACCCAGGC CTGTGATCCT GGACCCGGCG

                10         20         30         40         50
1001 GACCCTACAG GAAACTTGGG TGGTGGAGAC CCAAAGGGTT GGAGGCAGCT

                60         70         80         90        100
1051 GGCACAAGAG GCTGAGGCCT GGCTGAATTA CCCATGCTTT AAGAATTGGG

                10         20         30         40         50
1101 ATGGGTCCCC AGTGAGCTCC TGGATTCTGC TGGCTGAAAG CAACAGTACA

                60         70         80         90        100
1151 GACGATGAGA CCGACGATCC CAGGACGTAT CAGAAATATG GTTACATTGG

                10         20         30         40         50
1201 AACACATGAG TACCCTCATT TCTCTCATAG ACCCAGCACG CTCCAGGCAG

                60         70         80         90        100
1251 CATCCACCCC ACAGGCAGAA GAGGACTGGA CCTGCACCAT CCTCTGAATG

                10         20         30         40         50
1301 CCAGTGCATC TTGGGGGAAA GGGCTCCAGT GTTATCTGGA CCAGTTCCTT

                60         70         80         90        100
1351 CATTTTCAGG TGGGACTCTT GATCCAGAGA AGACAAAGCT CCTCAGTGAG

                10         20         30         40         50
1401 CTGGTGTATA ATCCAAGACA GAACCCAAGT CTCCTGACTC CTGGCCTTCT

                60         70         80         90        100
1451 ATGCCCTCTA TCCTATCATA GATAACATTC TCCACAGCCT CACTTCATTC

                10         20         30         40         50
1501 CACCTATTCT CTGAAAATAT TCCCTGAGAG AGAACAGAGA GATTTAGATA

                60         70         80         90        100
1551 AGAGAATGAA ATTCCAGCCT TGACTTTCTT CTGTGCACCT GATGGGAGGG

                10         20         30         40         50
1601 TAATGTCTAA TGTATTATCA ATAACAATAA AAATAAAGCA AATACCAAAA
```

FIG. 21

Human 2-5A-Synthetase Amino Acid Sequence

SEQ ID NO:11:

```
          10         20         30         40         50
1234567890 1234567890 1234567890 1234567890 1234567890
MMDLRNTPAK SLDKFIEDYL LPDTCFRMQI DHAIDIICGF LKERCFRGSS  50
YPVCVSKVVK GGSSGKGTTL RGRSDADLVV FLSPLTTFQD QLNRRGEFTQ 100
EIRRQLEACQ RERALSVKFE VQAPRWGNPR ALSFVLSSLQ LGEGVEFDVL 150
PAFDALGQLT GSYKPNPQIY VKLIEECTDL QKEGEFSTCG TELQRDFLKQ 200
RPTKLKSLIR LVKHWTQNCK KKLGKLPPQY ALELLTVYAW ERGSMKTHFN 250
TAQGFRTVLE LVINYQQLCI YWIKYYDFKN PIIEKYLRRQ LTKPRPVILK 300
PADPTGNLGG GDPKGWRQLA QEAEAWLNYP CFKNWDGSPV SSWILLAESN 350
STDDETDDPR TYQKYGYIGT HEYPHFSHRP STLQAASTPQ AEEDWTCTIL 400
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02058

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Nature, Vol. 330, issued 10 December 1987, Chebath et al., "Constitutive expression of (2'-5') oligo A synthetase confers resistance to picornavirus infection", pages 587-588, see the entire document.	1-3, 5-7, 9-16, 20-22, 24-26, 28-30, 32-36, 39-65, 84-86, 103, 104, 117, 118, 120-123, 125, 126, 129, 130, 132, 133, 136, 137, 139, 140, 143, 144, 145, 147
Y	Virology, Vol. 179, Issued 1990, Coccia et al., "A full-length murine 2-5A synthetase cDNA transfected into NIH-3T3 cells impairs EMCV but not VSV replication", pages 228-233, see the entire document.	1-3, 5-7, 9-16, 20-22, 24-26, 28-30, 32-36, 39-65, 84-86, 103, 104, 117, 118, 120-123, 125, 126, 129, 130, 132, 133, 136, 137, 139, 140, 143, 144, 145, 147
Y	Journal of Virology, Vol. 66, No. 10, issued October 1992, Meurs et al., "Constitutive expression of human double-stranded RNA-activated p68 kinase in murine cells mediates phosphorylation of eukaryotic initiation factor 2 and partial resistance to encephalomyocarditis virus growth", pages 5805-5814, see the entire document.	1, 4, 8, 12-20, 23, 27, 29-31, 37-49, 61-76, 94, 95, 118, 119, 121, 123, 127-129, 134-136, 141-143, 145, 148
Y	The EMBO Journal, Vol. 4, No. 7, Issued 1985, Saunders et al., "Human 2-5A synthetase: characterization of a novel cDNA and corresponding gene structure", pages 1761-1768, see the entire document.	1, 4, 5, 7-16, 24, 26, 28-30, 35, 36, 39-65, 97-118, 120-123, 125, 126, 129, 130, 132, 133, 136, 137, 139, 140, 143,144, 145, 147,

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02058

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 435/199, 240.2, 240.4, 252.3, 320.1; 536/23.5; 800/205
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/199, 240.2, 240.4, 252.3, 320.1; 536/23.5; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Hiatt, "Transgenic Plants, Fundamentals and Applications", published 1993 by Marcel Dekker, Inc. (N.Y.), pages 79-91, see the entire document.	1-76, 84-86, 94, 95, 103, 104, 117-149, 160, 161
Y	Dodds, "Plant Genetic Engineering", published 1987 by Cambridge University Press (N.Y.), pages 61-93, see the entire document.	1-76, 84-86, 94, 95, 103, 104, 117-149, 160, 161

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 25 MAY 1995	Date of mailing of the international search report 07 JUN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ERIC GRIMES Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02058

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Cell, Vol. 62, Issued 27 July 1990, Meurs et al., "Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon", pages 379-390, see the entire document.	1, 4, 8, 12-19, 23, 27, 29-31, 37-49, 61-76, 89-96, 108,110, 112,115, 116, 118, 119, 121, 123, 127-129, 134-136, 141-143, 148
Y	Cell, Vol. 72, issued 12 March 1993, Zhou et al., "Expression cloning of 2-5A-dependent RNAase: A uniquely regulated mediator of interferon action", pages 753-765, see the entire document.	1-3, 6, 9-14, 17-22, 25, 28, 29, 31-34, 39-60, 66-93, 96, 108, 110, 112, 115-126, 131-133, 138-140, 145, 146, 149-162
Y	Journal of Cellular Biochemistry, Supplement 16B, issued February 1992, Silverman et al., "Molecular cloning of 2-5A-dependent RNase: an endoribonuclease involved in interferon action", page 163, see abstract G520.	1-3, 6, 9-14, 17-22, 25, 28, 29, 31-34, 39-60, 66-93, 96, 108, 110, 112, 115-126, 131-133, 138-140, 145, 146, 149-162
Y	Journal of Biological Chemistry, Vol. 266, No. 9, Issued 25 March 1991, Salhzada et al., "Polyclonal antibodies against RNase L", pages 5808-5813, see the entire document.	1-3, 6, 9-14, 17-22, 25, 28, 29, 31-34, 39-60, 66-93, 96, 108, 110, 112, 115-126, 131-133, 138-140, 145, 146, 149-162
Y	Science, Vol. 222, Issued 18 November 1983, Young et al., "Yeast RNA polymerase II genes: isolation with antibody probes", pages 778-782, see the entire document.	1-3, 6, 9-14, 17-22, 25, 28, 29, 31-34, 39-60, 66-93, 96, 108, 110, 112, 115-126, 131, 133, 138-140, 145, 146, 149-162

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02058

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Biological Chemistry, Vol. 263, No. 15, issued 25 May 1988, Silverman et al., "Purification and analysis of murine 2-5A-dependent RNase", pages 7336-7341, see the entire document.	163
A	Journal of Interferon Research, Vol. 14, issued 1994, Silverman, "Fascination with 2-5A-dependent RNase: A unique enzyme that functions in interferon action", pages 101-103, see the entire document.	1-163
Y	The EMBO Journal, Vol. 12, No. 8, issued 1993, Hassel et al., "A dominant negative mutant of 2-5A-dependent RNase suppresses antiproliferative and antiviral effects interferon", pages 3297-3304, see the entire document.	1-3, 6, 9-14, 17-22, 25, 28, 29, 31-34, 39-60, 66-76, 84-86, 117-127, 131-134, 138-141, 145, 146
Y	Virology, Vol. 193, No. 2, issued April 1993, Lee et. al., "the interferon-induced double-stranded RNA-activated human p68 protein kinase inhibits the replication of vaccinia virus", pages 1037-1041, see the entire document.	1, 4, 8, 12-20, 23, 27, 29-31, 37-49, 61-76, 94, 95, 118, 119, 121, 123, 127-129, 134-136, 141-143, 145, 148

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02058

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02058

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01H 1/00, 3/00, 4/00; A01K 63/00; C12N 1/21, 5/04, 5/10, 9/22, 15/52, 15/54, 15/55, 15/63

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, Dialog (Medline, BIOSIS, Agricola, Derwent WPI, Derwent Biotechnology Abstracts)
search terms: 2-5A, RNAse, synthetase, PKR, dsRNA, kinase, RNAse L, antiviral, virus or viral, resistant or resistance, transgenic, plant, DNA or cDNA, vector

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 2, 3, 6, 21, 22, 25, 32-34 and 84-86, drawn to transgenic plants comprising a 2-5A-dependent RNase gene.
Group II, claims 5, 7, 24, 26, 35, 36, 103 and 104, drawn to transgenic plants comprising a 2-5A synthetase gene.
Group III, claims 4, 8, 23, 27, 37, 38, 94 and 95, drawn to transgenic plants comprising a PKR gene.
Group IV, claims 9-11, 28, 50-60, 117, 120 and 122, drawn to transgenic plants comprising a 2-5A-dependent RNase gene and a 2-5A synthetase gene.
Group V, claims 12-14, 29, 39-49, 118, 121 and 123, drawn to transgenic plants comprising a 2-5A-dependent RNase gene, a 2-5A synthetase gene and a PKR gene.
Group VI, claims 15, 16, 30 and 61-65, drawn to transgenic plants comprising a 2-5A synthetase gene and a PKR gene.
Group VII, claims 17-19, 31, 66-76 and 119, drawn to transgenic plants comprising a 2-5A-dependent RNase gene and a PKR gene.
Group VIII, claims 77-83, 87, 88 and 162, drawn to DNA, vectors and host cells comprising a 2-5A-dependent RNase gene.
Group IX, claims 89-93 and 96, drawn to vectors and host cells comprising a PKR gene.
Group X, claims 97-102, 105 and 106, drawn to vectors and host cells comprising a 2-5A synthetase gene.
Group XI, claims 107, 109, 111, 113 and 114, drawn to host cells comprising a 2-5A-dependent RNase gene, and a 2-5A synthetase gene.
Group XII, claims 108, 110, 112, 115 and 116, drawn to host cells comprising a 2-5A-dependent RNase gene, a 2-5A synthetase gene and a PKR gene.
Group XIII, claims 124, 131, 138 and 146, drawn to a method of making virus-resistant transgenic plants by transformation with DNA encoding 2-5A-dependent RNase.
Group XIV, claims 125, 126, 132, 133, 139 and 140, drawn to a method of making transgenic plants by tranforming with DNA encoding 2-5A-dependent RNase and DNA encoding 2-5A synthetase.
Group XV, claims 127, 134 and 141, drawn to a method of making virus-resistant transgenic plants by transforming with DNA encoding 2-5A-dependent RNase and DNA encoding PKR.
Group XVI, claims 128, 135, 142 and 148, drawn to a method of making virus resistant transgenic plants by transforming with DNA encoding PKR.
Group XVII, claims 129, 136 and 143, drawn to a method of making virus-resistant transgenic plants by transforming with DNA encoding PKR and DNA encoding 2-5A synthetase.
Group XVIII, claims 130, 137, 144 and 147, drawn to a method of making transgenic plants by transforming with DNA encoding 2-5A synthetase.
Group XIX, claims 149, 160 and 161, drawn to transgenic plants comprising 2-5A-dependent RNase antisense DNA.Group XX, claims150-159, drawn to vectors and host cells comprising 2-5A-dependent RNase antisense DNA.
Group XXI, claim 163, drawn to human 2-5A-dependent RNase.

Claims 1 and 20 are generic to Groups I-VIII and will be examined with the elected Group(s) to the extent they read thereon.

Claim 145 is generic to Groups XIII-XVIII and will be examined with the elected Group(s) to the extent it reads thereon.

The inventions listed as Groups I-XXI do not relate to a single inventive concept under PCT Rule 13.1 because, under

PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The claims of Group I have a technical feature of transgenic plants comprising a 2-5A-dependent RNase gene. The claims of Group II have a technical feature of transgenic plants comprising a transgenic plants comprising a 2-5A synthetase gene. The claims of Group III have a technical feature of transgenic plants comprising a PKR gene. The claims of Group IV have a technical feature of transgenic plants comprising a 2-5A-dependent RNase gene and a 2-5A synthetase gene. The claims of Group V have a technical feature of transgenic plants comprising a 2-5A-dependent RNase gene, a 2-5A synthetase gene and a PKR gene. The claims of Group VI have a technical feature of transgenic plants comprising a 2-5A synthetase gene and a PKR gene. The claims of Group VII have a technical feature of transgenic plants comprising a 2-5A-dependent RNase gene and a PKR gene. The claims of Group VIII have a technical feature of DNA encoding 2-5A-dependent RNase. The claims of Group XI have a technical feature of DNA encoding PKR. The claims of Group X have a technical feature of DNA encoding 2-5A-synthetase. The claims of Group XI have a technical feature of host cells comprising both a 2-5A-dependent RNase gene and a 2-5A synthetase gene. The claims of Group XII have a technical feature of host cells comprising a 2-5A-dependent RNase gene, a 2-5A synthetase gene and a PKR gene. The claims of Group XIII have a technical feature of transforming plants to virus-resistance with DNA encoding 2-5A-dependent RNase. The claims of Group XIV have a technical feature of transforming plants to virus-resistance with DNA encoding 2-5A-dependent RNase and DNA encoding 2-5A synthetase. The claims of Group XV have a technical feature of transforming plants to virus-resistance with DNA encoding 2-5A-dependent RNase and DNA encoding PKR. The claims of Group XVI have a technical feature of transforming plants to virus-resistance with DNA encoding PKR. The claims of Group XVII have a technical feature of transforming plants to virus-resistance with DNA encoding 2-5A synthetase and DNA encoding PKR. The claims of Group XVIII have a technical feature of transforming plants to virus-resistance with DNA encoding 2-5A synthetase. The claims of Group XIX have a technical feature of transgenic plants comprising 2-5A-dependent RNase antisense DNA. The claims of Group XX have a technical feature of 2-5A-dependent RNase antisense DNA. The claims of Group XXI have a technical feature of human 2-5A-dependentRNase.However, note that PKR, 2-5A-dependent RNase and 2-5A synthetase were each known in the prior art (see, e.g., the references on pages 2-6 of the description) and hence the various Groups of inventions do not share a technical relationship involving one or more of the same or corresponding "special technical features", i.e. those technical features that define a contribution which each invention, considered as a whole, makes over the prior art. They therefore do not fulfill the requirements of unity of invention and a holding of lack of unity for examination purposes is proper.